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By Gunnar Persson

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Cardiovascular Complications in Diabetics and Subjects with Reduced Glucose Tolerance

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Contents

Introduction

The present study is mainly focused on the occurrence of athero-sclerotic manifestations in diabetics and in individuals with reduced glucose tolerance followed during 9 years.

In 1962 the diabetes section of the Medical clinic at the University Hospital in Lund became interested in the prevalence of reduced glucose tolerance in the population and of the incidence of vascular complications in diabetes.

In a diabetes detection survey 82% of the population (228,833 individuals) were screened for glucosuria after a carbohydrate rich meal. Individuals with "borderline glucose tolerance" were identified with strict criteria. Some of the borderline subjects were randomly allocated to 4 different groups, given no treatment, dietary restrictions, dietary restrictions and placebo tablets, dietary restrictions and tolbutamide 1.5 g daily respectively. These borderline groups, and a group of control subjects with normal glucose tolerance have now been studied 9 years later with an exercise test and plethysmography. ECG reactions and plethysmographic variables have been related to the different types of treatment and to the presence of various so called "risk factors" for atherosclerosis, i.e. circumstances associated statistically with an increased tendency to cardiovascular morbidity and mortality.

In another study on patients with manifest diabetes, originally intended to compare their occupational adaptation with that of social twins, detailed hemodynamic observations were made in 1963. Most of these individuals have been re-examined 9 years later with an exercise test. ECG changes at rest and after exercise were related to the findings at the first examination and to the duration of diabetes, the degree of retinopathy and to risk factors for atherosclerosis.

The present study strongly supports that prevention of large vessel complications in diabetics and in individuals with reduced glucose tolerance is achieved not only by good control of blood glucose but also by attention to such factors as hypertension, hyperlipidemia and smoking.

The author

Exercise tests in male diabetics

A nine years follow-up study with special reference to ECG changes and cardiovascular morbidity

by G PERSSON

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ABSTRACT

Seventy-six male diabetics and 46 male controls studied with exercise tests in 1963 were examined 9 years later with a new test under similar conditions. Mean age in 1972 was 42 years in both groups.

Systolic blood pressure at rest was unchanged in the controls but had risen significantly in the diabetics especially in the smokers

During exercise systolic blood pressure was significantly higher in the diabetics than in the controls at all load levels. The differences were larger in 1972 than in 1963

In 1963 9% of the controls and 27% of the diabetics in 1972 26% of the controls and 50% of the diabetics exhibited pathological post-exercise S-T depressions. These S-T changes were more common in the smokers especially among the diabetics.

Thirty-six diabetics with normal post exercise ECG in 1972 (4 of them with pathological post-exercise S-T depressions in 1963) had significantly lower age cholesterol, triglycerides systolic and diastolic blood pressure as compared with diabetics with pathological post-exercise S-T depressions. In this pathological" group the frequency of smokers was higher and sedentary occupations dominated

There were 12 diabetics with clinical and/or electrocardiographic myocardial infarction 6 of them died from this cause There was 1 control subject with ECG sign of myocardial infarction. Only 1 of the 13 subjects with myocardial infarction was a non-smoker He had a long duration of diabetes, high blood pressure already in 1963 and high lipid levels.

INTRODUCTION

In several epidemiologic studies it has been reported that the risk of developing coronary heart disease can be predicted from i.a. age blood pressure serum cholesterol and smoking habits (2 20) The incidence of coronary heart disease is related also to other variables, such as relative body weight, physical activity and blood glucose levels (17 23 29 42) The connection between diabetes mellitus and heart disease has recently been reviewed by Scott, who emphasizes the magnitude of the problem (19). In diabetes the atherosclerotic process is accelerated leading to increased morbidity and mortality in e g myocardial infarction (28 33 41) In the U.S coronary artery disease accounts for more than half of the deaths in diabetic subjects with onset after the age of 20 (11).

Attempting early diagnosis of cardiovascular complications, Karlefors (26) performed circulatory studies in male diabetics without subjective symptoms of cardiovascular disease The main findings at a conventional exercise test were that the diabetics had higher heart rate and blood pressure and a higher frequency of pathological S-T and T changes in the electrocardiogram than a control group.

In the present study the patients in Karlefors series have been re-examined 8–10 years after the first investigation with respect to cardiovascular morbidity and changes in heart rate blood pressure working capacity and ECG changes The results have been correlated to different variables mentioned above

MATERIAL

Of the 84 male diabetics originally studied (26) 13 could not be re-examined the reasons being given in Table I The patients were divided by Karlefors into 3 groups with 0–4 5–14 and more than 14 years of duration of their diabetes Now 8–10 years later the patients in these groups have had diabetes for < 15 15–24 and > 24 years respectively All the diabetics re-examined have been treated with diet and insulin and regularly attended the Outpatient Clinic of the Department of Medicine With only few exceptions their diabetes was regarded as being under satisfactory control i.e no glucosuria blood glucose levels lower than 8.3 mMol/l prior to breakfast and post prandial levels of blood glucose below 11 1 mMol/l (1 24)

In 1963 76 age-matched controls were studied The controls were healthy normal men without symptoms or signs of cardiovascular or respiratory disease with normal blood glucose and normal urine tests and with no family story of diabetes The controls had been recruited at the Blood Donor Unit of the hospital at the Students Union and at the employment agency of the city In addition the Institute of Social Medicine at the hospital selected some control subjects as social twins to the diabetics

Of the 76 controls originally studied a representative subsample of 46 were re-examined 27 answered a questionnaire, 1 individual had health problems and 2 individuals could not be traced (Table I) The original study (26) took place in 1962–1964 and the follow up was made in 1971–1973 For simplicity the years of examination are given as 1963 and 1972 respectively

Table II shows that the mean values in 1963 for age heart rate blood pressure maximum load and S-T codings in the 46 re-examined controls do not differ from the corresponding values for those not re-examined

In comparing the participants of the follow up study we find similar distribution of age height and weight for diabetics and controls (Table III) Blood glucose values but not those of serum cholesterol and triglycerides were different in the two groups ($p < 0\ 001$) None of the controls had albuminuria or a raised serum creatinine Among the diabetics 10 had had constant albuminuria during the last years Five of these had slightly raised serum creatinine levels and 6 had advanced retinopathy in 1972

The occupations of the subjects are given in Table IV There is a preponderance of occupations that involve more physical activity in diabetics 54% against 28% in the controls ($p < 0\ 05$) This difference was not penetrated in 1963 but it obviously indicates that controls and diabetics are not fully comparable (26)

Table V shows that 41% of the controls and 58% of the diabetics were smokers. The difference was not significant ($p < 0\ 1$) In a previous study (34) of another group of subjects there were significantly more smokers among the diabetics Of the 1° ex smokers 10 had quit smoking between 1963 and 1972 Otherwise smoking habits were quite constant.

The observed difference in smoking habits made it necessary to divide the material further (Table V) Even then we find no differences in age height, cholesterol or triglycerides but non smoking diabetics are heavier than both smoking diabetics and control groups ($p < 0\ 05$)

TABLE I Participation of subjects in [illegible] follow-up study

Figures refer to the number of subjects

				Subjects not re-examined in 1972					
				Dead		Not healthy		Questionnaire [illegible]	
Group	Participants 1971	Re-examined 1972	Not re-examined	CHD*	Other causes	CHD*	Other causes	In good health	No response
Control	76	46	30	0	0	0	1	27	2
Diabetics	84	71	13	[illegible]	3	2	1	3	0

*CHD=coronary heart disease

TABLE II Data in 1971 of re-examined and not re-examined [illegible]

Mean values ± S.E.M.

			At rest			At maximum load			E-T [illegible]		[illegible]	
Group	No. of subjects	Age (years)	Heart rate (beats/min)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Heart rate (beats/min)	Systolic pressure (mm Hg)	Maximum load (W)	At rest: Pathological	At rest: Normal	Pathological	Normal
Re-examined	46	51.5 ± [illegible]	72.6 ± 1.71	133 ± 1.5	82 ± 1	72 ± 1	197 ± 2.9	177 ± 4.5	0	46		42
Not re-examined	30	38 ± 1.35	71 ± [illegible]	130 ± [illegible]	86 ± [illegible]	172 ± 1	198 ± 3.8	105 ± 5.4		30	3	27

TABLE III Description of the material [illegible] 1972

Mean values ± S

Group	No. of examined	Age (years)	Height (cm)	Weight (kg)	Blood glucose (mmol/l)	Cholesterol (mmol/l)	Triglycerides (mmol/l)
Controls	46	42 ± 1.90	77 ± 0.8	73 ± [illegible]	5 ± [illegible]	[illegible] 17	1.5 ± 0.22
Diabetics	71	42 ± [illegible]	170 ± 0.8	73 ± [illegible]	[illegible]	[illegible]	[illegible] 17

TABLE IV Type of occupation

Group	Predominantly sitting	Sitting or standing some walking	Walking some heavy lifting [illegible]	Heavy manual work
Control		27		2
Diabetics		22	37	

TABLE V [illegible]

[illegible] non-smokers were excluded from each group

Mean values ± [illegible]

Smoking habits	Group	No. of subjects	Age (years)	Height (cm)	Weight (kg)	Cholesterol (mmol/l)	Triglycerides (mmol/l)
Smokers	Control	19	[illegible]	[illegible]	72 ± [illegible]	[illegible]	[illegible]
	Diabetics	47	42 ± [illegible]	[illegible]	73 ± [illegible]	[illegible]	[illegible]
Non-smokers	Control	27	42 ± [illegible]	177 ± [illegible]	72 ± [illegible]	[illegible]	[illegible]
	Diabetics	24	41 ± [illegible]	176 ± [illegible]	76 ± [illegible]	[illegible]	[illegible]

METHODS

The examinations started at 8 o clock in the morning Eating and drinking apart from water were not allowed from 10 o clock p.m. the day before. The individuals were asked not to smoke on the day of examination Blood samples were collected for analysis of glucose, cholesterol triglycerides and potassium and a urine sample was tested for the presence of glucose or albumin Thereafter breakfast was allowed and the diabetics followed their usual routine with respect to dose of insulin and calory intake. Exercise tests were carried out as in the previous study 2–4 hours after the breakfast.

In the diabetics the degree of retinopathy was assessed by an eye-specialist and classified according to the following criteria as *no retinopathy" = 0"* i e no retinal vascular changes known to be associated with diabetes *"mild retinopathy" = "1" if there were one* or two microaneurysms and/or minor hemorrhages *moderate retinopathy" = "2"* if several microaneurysms and/or hemorrhages and/or exudates were present *severe retinopathy" = "3" with signs of proliferative* changes and/or if there were severe disturbances of vision

The individuals were asked about their smoking habits and divided into three groups *non-smokers* ex smokers and smokers

Each subject was classified into one of 4 occupational groups with regard to the physical activity involved (38 see Table IV) A similar classification as regards spare-time *physical activity was not feasible since it was* found difficult to assess this *retrospectively* Occupation on the other hand was *generally* the same over the observation period

All subjects were carefully examined by the same physician with special reference to the circulatory system. Heart rate and blood pressure were measured after 15 minutes of rest, during the exercise test and immediately 4 and 10 minutes after its end (26) A standard 13 cm wide cuff was used For reasons pointed out before (27) diastolic pressures during exercise were not analysed.

A standard 12 lead ECG (I II III aVR aVL, aVF V_1 V_2, V_3 V_4 V_5 V_7) was recorded on a 6-channel ink jet recorder (Elema-Schönander) before and immediately 4 and 10 minutes after exercise During exercise the indifferent electrode for precordial leads was moved *to the forehead and consequently CH leads were recorded*

ECG was recorded at rest both in recumbent position and sitting on the bicycle. The exercise test was done sitting on an electrically braked *bicycle ergometer in the same way as described by Karlefors* with the exception that the load was graded in watts (W) 50 watts approximately corresponding to the 300 kpm/min used *previously* (26) The bicycle ergometer used was calibrated *regularly*

Exercise started at 50 watts with determinations of heart rate, blood pressure breathing frequency and recording of ECG every other minute. Circulatory steady state was considered *to have been obtained* if the heart rate at a given work load *increased less than* 5 beats/min between 2 observations with 2 minutes interval If a steady state had been obtained the exercise load was increased by 50 watts and a new steady state was awaited *If steady state was not present after 4 minutes* of exercise *this was continued on the same* load with measurements every *other minute* until a steady state had been reached or work had to be stopped Exercise was stopped when subjective symptoms (e g excessive tiredness *and/or pain*) or increase in heart rate or *blood pressure or the occurrence* of ECG abnormalities made a *continuation inadvisable*

All ECGs were coded separately by an experienced ECG observer at the Department of Clinical Physiology and by a physician None of them knew whether the ECG be-

longed to a control or a diabetic subject. In those cases where the coding results of the two observers differed the tracings were discussed so that a unanimous opinion was reached (8) The tracings were classified according to a modified Minnesota code (9) The S-T depressions were coded as *ischemic* (horizontal or downward sloping) *slowly ascending* or *rapidly ascending* according to criteria given by Punsar and others (3 56 39). Since Punsar et al. found that both ischemic and slowly ascending S-T depressions were associated with increased morbidity in coronary heart disease these S-T changes were sometimes grouped together and referred to as pathological S-T changes The previous ECG tracings from 1962 to 1964 were all recoded in the same manner in order to be comparable. To obtain a quantitative estimate of T wave changes amplitudes of R and T waves were measured in lead V_5, and the T/R ratio was calculated (26)

For the statistical comparison between controls and diabetics Student's t-test and Chi^2 test were used. Conventional probability levels of significance were applied.

RESULTS

Heart rate and blood pressure at est (Tables VI–IX)

There was no significant difference in heart rate in 1965 between controls and diabetics, but in 1972 the heart rate was higher in the diabetics ($p < 0.001$)

The systolic blood pressure was already in 1965 higher in diabetics than in controls ($p < 0.01$) and has risen in the diabetics during the following 9 years ($p < 0.05$). In controls however the systolic pressure was unchanged through the years and consequently the difference in 1972 between controls and diabetics is still more marked ($p < 0.001$) (Table VI) The increase in systolic blood pressure was most pronounced in the groups with very long duration of diabetes and with more advanced retinopathy (Tables VII and VIII) The diastolic pressure in 1965 was higher in diabetics than in controls ($p < 0.01$) During the observation period there were no further changes in diastolic pressure in either group The pulse pressure is significantly higher in diabetics in 1972 ($p < 0.001$) which reflects the increase in systolic pressure during the 9 years

When individuals are grouped according to smoking habits there is no significant increase in heart rate in any group However the heart rate in 1972 was lower in non-smoking controls than in the other groups ($p < 0.05$) (Table IX) Only smoking diabetics have increased their systolic pressures significantly ($p < 0.01$).

In controls there is no increase in systolic blood pressure with age but in the oldest diabetic group (>45 years) the systolic pressure has increased, although not significantly ($p < 0.1$) from 1965 to 1972 In 1972 the systolic pressure in this oldest diabetic group is higher than in the corresponding control group and in the younger diabetics ($p < 0.001$)

Heart rate and blood pressure during and after exercise (Table X)

Neither controls nor diabetics have changed their heart rates during exercise significantly between 1965 and 1972. In 1972 the diabetics have higher heart rates than controls at 50 and 100 watts and at maximum load ($p < 0.001$) At 150 and 200 watts and 4 minutes after exercise there is no difference between controls and diabetics. The systolic blood pressure is higher in diabetics than in controls at all loads and 4 minutes after work both in 1965 and 1972 ($p < 0.001$). The difference is even more pronounced in 1972 The systolic pressure 4 minutes after work is almost the same as that at rest, both in controls and in diabetics.

METHODS

The examinations started at 8 o clock in the morning Eating and drinking apart from water were not allowed from 10 o clock p m. the day before The individuals were asked not to smoke on the day of examination Blood samples were collected for analysis of glucose cholesterol triglycerides and potassium and a urine sample was tested for the presence of glucose or albumin Thereafter breakfast was allowed and the diabetics followed their usual routine with respect to dose of insulin and calory intake Exercise tests were carried out as in the previous study 2–4 hours after the breakfast.

In the diabetics the degree of retinopathy was assessed by an eye specialist and classified according to the following criteria as *no retinopathy*" = 0 i.e no retinal vascular changes known to be associated with diabetes "*mild retinopathy*" = 1 if there were one or two microaneurysms and/or minor hemorrhages *moderate retinopathy*" = "2" if several microaneurysms and/or hemorrhages and/or exudates were present "*severe retinopathy*" = 3" with signs of proliferative changes and/or if there were severe disturbances of vision

The individuals were asked about their smoking habits and divided into three groups non-smokers ex smokers and smokers

Each subject was classified into one of 4 occupational groups with regard to the physical activity involved (38 see Table IV) A similar classification as regards spare-time physical activity was not feasible since it was found difficult to assess this retrospectively Occupation on the other hand was generally the same over the observation period

All subjects were carefully examined by the same physician with special reference to the circulatory system Heart rate and blood pressure were measured after 15 minutes of rest during the exercise test and immediately 4 and 10 minutes after its end (26) A standard 13 cm wide cuff was used. For reasons pointed out before (27) diastolic pressures during exercise were not analysed

A standard 12 lead ECG (I II III aVR, aVL aVF V_1 V_2, V_3 V_4 V_5, V_7) was recorded on a 6-channel ink jet recorder (Elema Schönander) before and immediately 4 and 10 minutes after exercise During exercise the indifferent electrode for precordial leads was moved to the forehead and consequently CH leads were recorded

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Exercise started at 50 watts with determinations of heart rate blood pressure breathing frequency and recording of ECG every other minute. Circulatory steady state was considered to have been obtained if the heart rate at a given work load increased less than 5 beats/min between 2 observations with 2 minutes interval If a steady state had been obtained the exercise load was increased by 50 watts and a new steady state was awaited If steady state was not present after 4 minutes of exercise this was continued on the same load with measurements every other minute until a steady state had been reached or work had to be stopped Exercise was stopped when subjective symptoms (e g excessive tiredness and/or pain) or increase in heart rate or blood pressure or the occurrence of ECG abnormalities made a continuation inadvisable.

All ECGs were coded separately by an experienced ECG observer at the Department of Clinical Physiology and by a physician None of them knew whether the ECG be-

longed to a control or a diabetic subject. In those cases where the coding results of the two observers differed, the tracings were discussed so that a unanimous opinion was reached (8) The tracings were classified according to a modified Minnesota code (9). The S-T depressions were coded as *ischemic* (horizontal or downward sloping) *slowly ascending* or *rapidly ascending* according to criteria given by Punsar and others (3 36 39) Since Punsar et al. found that both ischemic and slowly ascending S-T depressions were associated with increased morbidity in coronary heart disease, these S-T changes were sometimes grouped together and referred to as "pathological S-T changes The previous ECG tracings from 1962 to 1964 were all recoded in the same manner in order to be comparable. To obtain a quantitative estimate of T wave changes, amplitudes of R and T waves were measured in lead V_6, and the T/R ratio was calculated (26)

For the statistical comparison between controls and diabetics Student's t-test and Chi²-test were used. Conventional probability levels of significance were applied

RESULTS

Heart rate and blood pressure at rest (Tables VI–IX)

There was no significant difference in heart rate in 1963 between controls and diabetics, but in 1972 the heart rate was higher in the diabetics ($p < 0.001$)

The systolic blood pressure was already in 1963 higher in diabetics than in controls ($p < 0.01$) and has risen in the diabetics during the following 9 years ($p < 0.05$) In controls, however the systolic pressure was unchanged through the years and consequently the difference in 1972 between controls and diabetics is still more marked ($p < 0.001$) (Table VI). The increase in systolic blood pressure was most pronounced in the groups with very long duration of diabetes and with more advanced retinopathy (Tables VII and VIII). The diastolic pressure in 1963 was higher in diabetics than in controls ($p < 0.01$) During the observation period there were no further changes in diastolic pressure in either group The pulse pressure is significantly higher in diabetics in 1972 ($p < 0.001$) which reflects the increase in systolic pressure during the 9 years

When individuals are grouped according to smoking habits there is no significant increase in heart rate in any group However the heart rate in 1972 was lower in non-smoking controls than in the other groups ($p < 0.05$) (Table IX). Only smoking diabetics have increased their systolic pressures significantly ($p < 0.01$)

In controls there is no increase in systolic blood pressure with age, but in the oldest diabetic group (>45 years) the systolic pressure has increased although not significantly ($p < 0.1$) from 1963 to 1972 In 1972 the systolic pressure in this oldest diabetic group is higher than in the corresponding control group and in the younger diabetics ($p < 0.001$)

Heart rate and blood pressure during and after exercise (Table X)

Neither controls nor diabetics have changed their heart rates during exercise significantly between 1963 and 1972. In 1972 the diabetics have higher heart rates than controls at 50 and 100 watts and at maximum load ($p < 0.001$) At 150 and 200 watts and 4 minutes after exercise there is no difference between controls and diabetics. The systolic blood pressure is higher in diabetics than in controls at all loads and 4 minutes after work both in 1963 and 1972 ($p < 0.001$). The difference is even more pronounced in 1972. The systolic pressure 4 minutes after work is almost the same as that at rest, both in controls and in diabetics.

TABLE VI Heart rate and blood pressure at rest.

Mean values ± S.E.M.

Group	No of subjects	Heart rate (beats/min) 1963	1972	Systolic pressure (mm Hg) 1963	1972	Diastolic pressure (mm Hg) 1963	1972	Pulse pressure (mm Hg) 1963	1972
Controls	48	72.6 ± 1.71	69.2 ± 1.88	133 ± 1.9	134 ± 2.1	82 ± 1.4	85 ± 1.8	52 ± 1.8	50 ± 1.7
Diabetics	71	75.0 ± 1.44	78.9 ± 1.46	141 ± 2.1	149 ± 2.6	88 ± 1.3	87 ± 1.0	53 ± 1.6	61 ± 2.0

TABLE VII Duration of diabetes in relation to blood pressure

Mean values ± S.E.M.

Duration of diabetes (years)	No of subjects	Systolic pressure (mm Hg) 1963	1972	Diastolic pressure (mm Hg) 1963	1972
< 15	36	138 ± 3.0	144 ± 3.5	85 ± 1.8	86 ± 1.4
15 - 24	22	146 ± 3.6	149 ± 4.1	89 ± 2.4	87 ± 1.5
> 24	13	144 ± 4.3	159 ± 7.4	94 ± 2.4	92 ± 2.4

TABLE VIII Blood pressure in relation to eye complications and duration of diabetes

Mean values ± S.E.M.

Retinopathy	No of subjects	Duration of diabetes (years)	Systolic pressure (mm Hg) 1963	1972	Diastolic pressure (mm Hg) 1963	1972
0	17	10.3 ± 0.89	136 ± 3.5	145 ± 4.8	84 ± 2.8	88 ± 2.2
1	24	15.0 ± 1.35	138 ± 4.1	148 ± 3.6	85 ± 2.0	85 ± 1.3
2	15	20.3 ± 1.79	143 ± 4.8	147 ± 5.1	90 ± 2.4	86 ± 1.6
3	12	23.8 ± 3.04	149 ± 4.2	160 ± 8.1	96 ± 2.0	92 ± 2.7

TABLE IX Heart rate and blood pressure at rest, in relation to smoking habits. Ex-smokers excluded.

Mean values ± S.E.M.

Smoking habits	Group	No of subjects	Heart rate (beats/min) 1963	1972	Systolic pressure (mm Hg) 1963	1972	Diastolic pressure (mm Hg) 1963	1972
Smokers	Control	19	77.8 ± 1.86	75.4 ± 3.22	134 ± 2.5	136 ± 2.8	81 ± 1.8	86 ± 1.6
	Diabetics	41	76.6 ± 1.80	81.4 ± 1.96	137 ± 2.4	151 ± 3.5	86 ± 1.5	87 ± 1.3
Non-smokers	Control	21	69.7 ± 3.09	65.1 ± 2.48	135 ± 2.7	134 ± 3.6	84 ± 2.2	84 ± 1.6
	Diabetics	24	70.4 ± 2.18	75.2 ± 3.37	142 ± 3.4	146 ± 4.4	89 ± 2.3	89 ± 1.7

The heart rates at all load levels are higher in smokers than in non-smokers both in controls and in diabetics. The systolic pressure is the same in smoking controls as in non-smoking controls. In the diabetics however the smoking individuals have significantly higher systolic pressure than the non-smoking ones in 1972. This difference was not seen in 1963.

Thus in this material controls have not changed their heart rates or blood pressure reactions at rest or during exercise from 1963 to 1972. The diabetics, on the other hand have increased their blood pressures and the differences between controls and diabetics have become highly significant both at rest and during exercise. It is mainly the smoking diabetics who have increased their systolic pressures.

Physical working capacity (Table XI)

Heart rate at maximum load has not changed from 1963 to 1972 in either group. Maximum load however has risen in controls independent of smoking habits ($p < 0.001$). The slight increase of maximum load in diabetics, however was not significant. Consequently in 1972 there are significantly higher maximum load values in controls than in corresponding diabetic groups ($p < 0.01$ respectively $p < 0.05$). Also the mean load during the last 4 minutes of exercise differed between the controls and the diabetics, but less than the maximum load. In 1972 the individuals appear to have been pressed to higher load levels. Thus in 1963 8% of the diabetics and 20% of the controls reached a steady state at 200 watts whereas in 1972 10% and 50% respectively achieved this.

The non-smoking groups have higher load values than corresponding smoking groups ($p < 0.05$).

ECG reactions at rest and 4 minutes after exercise (Tables XII–XV)

In 1963 at rest, before exercise, there were no S-T abnormalities in the controls and only a few ones in the diabetics (6%). In 1972 there were three controls (6.5%) and 12 diabetics (17%) with S-T changes. These pathological changes were more frequent among the smokers. Thus 9 of 41 smoking (22%) but only 2 of 24 non-smoking diabetics (8%) had ischemic or slowly ascending S-T depressions in 1972 (Table XII).

The frequency of S-T changes at rest was not related to the duration of diabetes or the degree of retinopathy. However the small number of patients with ECG abnormalities invalidates the analysis.

Especially during but also immediately after work S-T changes may be difficult to evaluate due to technical problems (26). Therefore special attention was given to S-T changes, R waves, T waves and T/R ratios 4 minutes after work. Lead V was used for analysis. In 1963 4 controls (9%) and 19 diabetics (27%) exhibited pathological post exercise S-T depressions, i.e. ischemic and slowly ascending S-T segments. In 1972 12 controls (26%) and 35 diabetics (50%) had pathological S-T changes 4 minutes after work (Fig. 1, Table XIII). The difference in the frequency of pathological S-T changes was significant between controls and diabetics both in 1963 and 1972 ($p < 0.01$). This difference had probably been even more pronounced if the total material from 1963 had been re-examined because of the high frequency of pathological post-exercise S-T depressions among those 15 diabetics who were not re-examined.

In non-smokers pathological post-exercise changes were found in 5% of the controls and 8% of the diabetics in 1963. In 1972 24% of the controls and 33% of the diabetics had pathological S-T changes 4 minutes after work. Pathological S-T changes were more

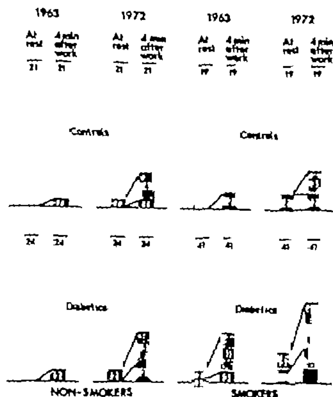


FIG. 1. S-T depressions (%) in 1963 and 1972 in relation to smoking habits

■ = ischemic

▩ = slowly ascending S-T segment

common among the smokers In 1963 10% of the smoking controls and 34% of the smoking diabetics and in 1972 26% of the smoking controls and 56% of the smoking diabetics have pathological S-T changes 4 minutes after exercise (Table XIII)

Pathological S-T changes 4 minutes after work became more frequent in 1972 in patients with long duration of diabetes and with advanced retinopathy Also a constant albuminuria during the last years was associated with S-T changes Thus of 10 diabetics with albuminuria 5 had pathological post-exercise S-T changes in 1963 and 8 in 1972

In order to study the development of S-T changes in 1972 in relation to the post exercise S-T codings of 1963 the individuals were divided in three groups with slowly ascending rapidly ascending and with no S-T depressions respectively (Table XIV).

Sixteen diabetics had slowly ascending S-T depressions 4 minutes after work in 1963 In 1972 4 of these had pathological S-T changes already at rest and 12 (75%) 4 minutes after work Eleven of them were smokers (69%) Seventeen diabetics had rapidly ascending S-T depressions 4 minutes after exercise in 1963 In 1972 2 of these had pathological S-T changes at rest and 8 (47%) 4 minutes after work. Ten of them were smokers (59%) Thirty five diabetics had normal S-T 4 minutes after work in 1963 In 1972 4 of these had pathological S-T changes at rest and 12 (34%) 4 minutes after work. Seventeen of them were smokers (49%)

In order to correlate the ECG reactions 4 minutes after work in 1972 with clinical data and with previous ECG reactions the diabetics have been grouped as follows Normal S-T in 1963 and 1972 (N N) pathological S-T depressions in 1963 but normal in 1972 (P N) normal S-T in 1963 but pathological S-T depressions in 1972 (N P) and pathological S-T depressions both in 1963 and 1972 (P P) (Table XV)

The two groups with normal S-T reactions in 1972 are very like each other as concerns different clinical data In the same manner the two groups with pathological S-T depressions in 1972 are very like each other

In the two groups with pathological ECG reactions in 1972 there is an increase in systolic pressure between 1963 and 1972 ($p < 0.1$) In the other two groups the systolic pressure is unchanged

In the two normal groups we find significantly lower mean values for age ($p < 0.01$) cholesterol ($p < 0.001$) triglycerides ($p < 0.05$) systolic pressure ($p < 0.01$) and diastolic pressure ($p < 0.05$) but weight blood sugar heart rate and daily consumption of tobacco (among the smokers) are not significantly different from the two "pathological" groups

TABLE X. Heart rate and systolic blood pressure at rest and during exercise

Mean values ± S.E.M.

Exercise load (W)	Group	Heart rate (beats/min) 1963	1972	Systolic pressure (mm Hg) 1963	1972	No. of subjects 1963	1972
50 steady state	Control	102 ± 2.2	97.1 ± 2.2	140 ± 2.8	143 ± 2.3	46	46
	Diabetics	107 ± 1.9	108 1.8	142 ± 2.0	160 ± 2.6	69	63
100 steady state	Controls	127 ± 2	119 ± 2.1	160 ± 2.4	163 ± 2.5	46	45
	Diabetics	136 ± 2	132 ± 2.2	184 ± 3.2	187 ± 3.8	69	66
150 steady state	Controls	146 ± 2.9	140 ±	198 ± 3.0	84 3	1	42
	Diabetics	154 ± 3.1	151 ± 2.6	203 2	208 ± 4.8	41	37
200 steady state	Controls	147 ± 5.0	160 3.2	21 ±	98 ± 3.4	9	33
	Diabetics	150 ± 6.2	167 ± 5.2	214 6.3	222 ± .2	6	11
Maximum load	Controls	172 ± 7	177 ± 1	197 2.9	202 ± 2.7	46	46
	Diabetics	167 ± 2.1	160 ± .9	21 ± 3.2	216 ± 2	71	71
6 minutes after exercise	Control	96 ± 1	90 ± 1	38 ±	38 ± 2.2	46	40
	Diabetics	98 ± 1	103 ±	143 ± 2.0	90 ± 2	71	73

TABLE XI Maximum load, mean load during the last 6 minutes, and heart rate at maximum load relative to smoking habits.

Mean values [illegible]

Smoking habit	Group	No. of subjects	Maximum load (W) 1963	1972	Mean load (W) during the last [illegible] minutes 1963	1972	Heart rate (beats/min) at maximum load 1963	1972
Smokers	Control	19	187	9 0	164 5	186 ± 7.3	75 ± 2	181 2
	Diabetics	4	150 ± 5.0	16 ±	144 ± 6	148 5	167 2	165 ± 3.1
Ex-smokers	Control	6	78 1 2	213 ± 10.5	75 ±	81 ± 9	172	177 ± 8
	Diabetics		42 ± 3	16 14	133 ± 8.3	158 ± 9	171 ± 4.9	165 8
Non-smokers	Control	21	186 ± 6	21 ± 8	180	201 ± 8	180 ± 2	174 ± 2.7
	Diabetics	14	73 ± 2	188 ± 9.7	166 6	178 ± 8	167 ± 8	79 3.8

TABLE XII [illegible] relative to smoking habits

Smoking habit	Group	ECG at rest in 1963: Ischaemic	Slow ascending	Rapidly ascending	No depression	Other (pathological) ( )	ECG at rest in 1972: Ischaemic	Slow ascending	Rapidly ascending	No depression	Pathological ( )
Smokers	Controls				14		2		2	14	11
	Diabetics			5	33			5	2	30	22
Non-smokers	Controls		6		13	0	0	1	2	18	5
	Diabetics		6		24		0	2	3	19	
Ex-smokers	Control			9	37	0	2	1	4	30	6 5
	Diabetics	1		3	48	6		0	5	54	

In the two groups with abnormal ECG in 1972 the frequency of smokers is higher than in the normal groups and sitting and standing occupations dominate. In the two groups with normal ECG most of the patients have occupations involving more physical work

The controls have also been grouped in the same way as mentioned above but the groups are too small to allow any conclusions However the tendency is the same as mentioned above

The frequency of ischemic and slowly ascending S-T depressions was studied in controls and diabetics grouped according to occupation The age distribution and diastolic pressure are similar in the different groups but the systolic pressure is higher in diabetics with sitting or standing occupation ($p < 0.05$) The number of individuals having very light and very heavy occupation is small Combining the two lighter occupational groups we get an incidence of pathological S-T changes of 30 and 67% in the controls and diabetics respectively In the combined groups with heavy occupation the corresponding figures are 8 and 34% in controls and diabetics respectively The higher frequency of pathological S-T depressions in the two lighter occupational groups can probably not be explained only by differences in blood pressures or smoking habits

The amplitudes of R and T waves at rest and 4 minutes after work are higher in controls than in diabetics both in 1963 and 1972 (R $p < 0.01$ T $p < 0.05$)

Both in controls and in diabetics R and T waves at rest and 4 minutes after exercise have lower amplitudes in 1972 compared with 1963 ($p < 0.01$)

In all groups both in 1963 and 1972 and independent of smoking habits there are higher T amplitudes immediately after exercise as compared with the resting amplitudes ($p < 0.05$–0.01) and with T amplitudes 4 minutes after exercise ($p < 0.01$)

The T amplitudes and the T/R ratios 4 minutes after exercise in individuals with pathological post-exercise S-T depressions significantly lower than corresponding in individuals with normal post-exercise ECG ($p < 0.001$) This points at a connection between low T amplitudes and T/R ratios on the one hand and ischemic and slowly ascending S-T depressions on the other hand In view of the probably more intimate relation between S-T changes and myocardial perfusion a detailed analysis of T wave amplitudes and T/R ratios is not presented here

Cardiovascular morbidity (Tables XVI–XVII)

In 1963 none of the controls or diabetics had electrocardiographic signs of infarction (Q-QS-complexes or abnormal R progression) In 1972 one of the 46 re-examined controls had an abnormal QS-complex. Of the 71 re-examined diabetics 3 had QS-complexes and one had abnormal R progression indicative of myocardial infarction. None of the controls has died in cardiovascular disease. Of 30 not re-examined controls 27 declared themselves to be in good health and without cardiovascular symptoms (Table I)

Of the 13 not re-examined diabetics 8 have sustained a myocardial infarction and 6 of these died from this cause (Table I) Table XVI shows that these 8 diabetics already in 1963 had higher systolic blood pressure, a longer duration of their diabetes and advanced retinopathy

That pathological post-exercise S-T reactions have a prognostic value is shown by the fact that most cases of myocardial infarction were found in individuals with pathological S-T reactions already in 1963 (Table XVII) Of 29 diabetics with pathological S-T reactions in 1963 9 (31%) sustained a myocardial infarction The incidence in 55 diabetics with normal ECG reaction in 1963 was only 7% (4 patients) the difference being significant ($p < 0.05$) The total incidence of probable myocardial infarctions is thus 12 out of 84

TABLE XIII S-T depression [illegible] minutes after exercise in relation to smoking habits

Smoking habits	Group	S-T [illegible] after exercise in 1963: Ischaemic	Slow ascending	Rapidly ascending	No depression	Pathological (%)	S-T [illegible] minutes after exercise in 1972: Ischaemic	Slowly ascending	Rapidly ascending	No depression	Pathological ( )
Smokers	Control	2	0	4	13	10	2	3	5	9	24
	Diabetics	3	11	15	17	34	10	3		14	56
Non-smokers	Control	1		5	15	5	1		5	11	24
	Diabetics	6	2	8	17	8	3	8	6	10	33
Total [illegible]	11 controls	3	1	3	29	9	3	9	1	21	25
	11 diabetics	3	16		35	27	14	21	11	28	90

TABLE XIV [illegible] at rest and [illegible] minutes after exercise in relation [illegible] minutes after exercise,

S-T [illegible] after exercise in 1963	Group	No. of subjects	S-T at rest in 1972: Ischaemic	Slowly ascending	Rapidly ascending	No depression	S-T [illegible] min after exercise in 1972: Ischaemic	Slow ascending	Rapidly ascending	No depression	Smoking habits: Non-smokers	Ex-smokers	Smokers
Slow ascending	Control		0	6	0		0		0		0	1	0
	Diabetics	14		2	6	2		8	0		2	3	1
Rapidly ascending	Control	13	0		3	10	0	1	8		5		
	Diabetics		0			12		5	3		8	2	10
No depression	Control	29	0			27	1		6	19	15	1	13
	Diabetics	35				29		8	0	15		1	17

TABLE XV [illegible] minutes after exercise between 1963 and 1972 in diabetic patients [illegible] [illegible] and other data

[illegible] are given for observations made in 1972

S-T reaction 1963	1972	No. of subjects	Age (years)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Systolic pressure (mm Hg) at rest 1963	1972	Diastolic pressure (mm Hg) at rest 1963	1972	Smoking habits: Non-smokers	Ex-smokers	Smokers
—		32	50 ± 25		± 07	30-2	140-2	86	85-1.0	15		16
path			54 ± 63 5	85 6	25	125 ± 2	125 ± 3	84-1.8	85 ± 3 9	2	0	2
		28	46 70	63 ± 1		146-4 9	100-3 3	92 ± 2 5	90-2		2	1
		15	± 3 09	64	± 09	142-4	156-6	92 ± 2	91 ± 2 8		2	12

S-T reaction 96	72	No. of subjects	[illegible]
—		32	15
path			
		23	6
		15	6

[illegible] ECG pathologic S- reaction

[illegible]

TABLE XVI Data of 1963 of diabetics in relation to development of myocardial infarction.

Mean values ± S.E.M.

Group	No of subject	Age (years)	Duration (years)	Retinopathy 0	1	2	3	At rest: Heart rate (beats/min)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	At maximum load: Heart rate (beats/min)	Systolic pressure (mm Hg)	Maximum load (W)
Re-examined													
Total	71	33 1	16 4	17	24	15	12	75	141	80	167	211	159
		1 09	–0 95					±1 4	–2 1	1 3	–2 1	3 2	3.8
	4	34 0	16 5		1	2	1	81	169	99	145	244	138
		–4 20	3 95					–9 3	7 7	±4 3	11 9	22 8	±12 5
No re-examined													
Total	13	31 8	22	1	2	2	8	74	153	90	170	223	154
		2 31	3 50					2 4	7.2	–4 4	±1 6	–9 3	±6 3
1 *	8	36 5	24			1	7	73	167	94	163	234	150
		±2 49	3 65					±3.2	10 8	5 0	±4 7	12 2	±9 5

Group	No of subjects	S T changes At rest: Pathological	Normal	4 min after exercise: Pathological	Normal	Pathological 4 min after exercise (%)
Re-examined						
Total	71	4	67	19	52	27
m.	4	1	3	1	3	25
Not re-examined						
Total	13	2	11	10	3	77
m.i *	8	1	7	8	0	100

* m.i myocardial infarction

TABLE XVII Post-exercise S-T reaction as prognostic factor

Group	S-T reaction 4 min after exercise in 1963	S-T reaction 4 min after exercise in 1972: Normal	Pathological	Subjects not examined in 1972: Dead: CHD*	Other cause	Not healthy: CHD*	Other cause	Questionnaires: In good health	No response	CHD+M (%)
Diabetics	Normal (88)	32(2m.i)	20(1 m.i)	0	1	0	1	1	0	7
	Pathological (29)	4	15(1 m.i)	6	0	2	0	2	0	31
Control	Normal (69)	34(1 m.i)	8	0	0	0	1	24	2	2
	Pathological (7)	0	4	0	0	0	0	3	0	0

* CHD myocardial infarction, coronary heart disease

diabetics (6 dead 2 clinical infarctions and 4 with ECG signs). One out of 46 re-examined controls has ECG signs of myocardial infarc tion.

It is seen that the infarctions occurred mainly in individuals with long duration of diabetes, advanced retinopathy high blood pressure sedentary occupation and in smokers.

DISCUSSION

During the 9 years the heart rate and blood pressure values have remained at nearly the same level in the controls. In the diabetics, on the other hand, both heart rate, systolic pressure and pulse pressure have risen The increased resting heart rate may be due to e g a diabetic autonomic neuropathy with impairment of vagal innervation of the heart (44) The rise in systolic pressure both at rest and during exercise may be due to more advanced arterial changes e.g medial sclerosis and/or atherosclerosis but also to a diabetic hypertension with influence on the renin-angiotensin system (15) or to changes in autonomic nervous function. The increase in systolic pressure appears predominantly in the smoking diabetics, but the ag of the patients the duration of diabetes and the presence of advanced retinopathy a e obviously also of importance

The maximum exercise load has increased between 1963 and 1972 with about 30 watts in the controls and with about 10–15 watts in the diabetics ex smokers not included. In n rmal subjects during the same period of time the working capacity is expected to decrease with about 20 watts due to age (37) Ther are several explanations to the higher alues now The most important one is probably that the subjects in 1963 were stopped before eaching their maximal capacity This would explain why the heart rate at maximum load has increased with about 5 beats/min. in the controls between 1963 and 1972 In normal subjects the maximal heart rate obtainable should be expected to decrease by at least 10 beats/min in 9 years (4) Thus the gap between the maximal heart rate recorded at the test and the predicted maximal heart rate is smaller in 1972 than in 1963

It is also possible that some subjects have increased their physical fitness Thus the greatest increase in maximum load is seen in ex smokers 58 watts in the controls and 25 watts in the diabetics One explanation for this may be that 10 of the 12 ex-smokers have stopped smoking after the first exercise test. Their maximum load values in 1963 corresponded rather well with those of other smokers this year The fact that they have stopped smoking during the 9 last years may have increased their physical fitness. This category may also have a greater interest of being in good physical fitness (37)

It is unlikely that technical changes could explain the differences, since the work tests were performed under similar conditions and with regularly calibrated bicycle ergometers.

It is generally agreed that certain S-T changes in connection with exercise tests may be a sign of non-symptomatic coronary heart disease (3 10 35) However a normal ECG after a maximal exercise test is of course no guarantee that severe coronary heart disease does not exist and that a subject will not sustain myocardial infarction (16) It was hoped by Karlefors (26) that the abnormal S-T changes noted by him in 1963 in diabetics, would be followed up by observations of future development of ECG changes and correlation to clinical data.

Both in 1963 and 1972 there are 2 to 3 times as many abnormal S-T depressions 4 minutes after exercise in the diabetics as in the controls. In 1963 9% and in 1972 26% of the controls have ischemic or slowly ascending S-T depressions These figures correspond rather well with several investigations on normal subjects of corresponding age (4 10

TABLE XVI Data in 1963 of diabetics in relation to development of myocardial infarction.
Mean values ± S.E.M.

Group	No. of subjects	Age (years)	Duration (years)	Retinopathy 0	1	2	3	At rest: Heart rate (beats/min)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	At maximum load: Heart rate (beats/min)	Systolic pressure (mm Hg)	Maximum load (W)
Re-examined													
Total	71	33.1 ± 1.09	16.4 ± 0.95	17	24	15	12	75 ± 1.4	141 ± 2.1	88 ± 1.3	167 ± 2.1	211 ± 3.2	159 ± 3.8
m.i.	4	34.0 ± 4.20	16.5 ± 3.95		1	2	1	81 ± 9.3	159 ± 7.7	99 ± 4.3	145 ± 11.9	244 ± 27.0	138 ± 12.5
Not re-examined													
Total	13	31.8 ± 2.37	22 ± 2.60	1	2	2	8	74 ± 2.4	153 ± 7.2	90 ± 4.4	170 ± 7.8	223 ± 9.3	154 ± 8.3
m.i.*	8	36.5 ± 2.49	24 ± 3.65			1	7	73 ± 3.2	161 ± 10.5	94 ± 5.0	183 ± 4.7	234 ± 12.2	150 ± 9.6

Group	No. of subjects	S-T dig At rest: Pathological	Normal	4 min after exercise: Pathological	Normal	Pathological 4 min after exercise (%)
Re-examined						
Total	71	4	67	19	52	27
m.i.	4	1	3	1	3	25
Not re-examined						
Total	13	2	11	10	3	77
m.i.*	8	1	7	8	0	100

* m.i. myocardial infarction

TABLE XVII Post-exercise S-T reaction as prognostic factor

Group	S-T reaction 4 min after exercise in 1963	S-T reaction 4 min after exercise in 1972: Normal	Pathological	Subjects not examined in 1972: Dead: CHD*	Other cause	Not able: CHD*	Other cause	Questionnaire: Good health	No response	CHD* (%)
Diabetics	Normal (55)	32(3 m.i.)	20(1 m.i.)	0	1	8	1	1	8	7
	Pathological (20)	4	15(1 m.i.)	4	8	2	0	2	8	31
Control	Normal (69)	34(1 m.i.)	8	0	0	8	1	24	2	2
	Pathological (7)	0	4	0	0	0	0	3	0	0

* m.i. myocardial infarction
* CHD coronary heart disease

have a longer duration of diabetes. The observed lower frequency of pathological post-exercise ECG changes in individuals with occupations involving physically heavy work is obviously related to the general relation between physical inactivity and coronary heart disease (e.g. 12, 25, 35, 40, 45).

A detailed analysis of physical spare-time activity and of dietary habits, including coffee drinking and alcoholic habits was not attempted because of variations through the years and because of difficulties in getting objective data. However just as Karlefors we have found that all individuals were regular coffee drinkers and without apparent alcoholic problems.

Blood glucose values before and after exercise were measured, but in agreement with Karlefors (26) they were not related to the ECG reaction, either in controls or in diabetics. However the mean blood glucose values are of course higher in the diabetics and it cannot be excluded that changes in myocardial glucose metabolism may influence the ECG. As mentioned above most of the diabetics were in good control but this means only that we have information about urine and blood glucose levels and lipids at isolated times in the life of diabetics. Variations during the day of blood glucose and other metabolic products e.g blood lactate ketone bodies, and hormones, e g growth hormone glucagon and catecholamines may be more important for the development of diabetic complications (1 24).

All but one of the 12 diabetics with clinical and/or ECG signs of myocardial infarction were smokers all but one had high blood pressure and retinopathy in 1963 Ten of them had a long duration of diabetes and had developed hypertension through the years. Eight of these 12 diabetics had pathological S-T changes already in 1963 The only non-smoking diabetic patient with myocardial infarction had a long duration of diabetes (17 years) high blood pressure already in 1963 and high blood lipid levels. The only control subject with ECG signs of infarction is a heavy smoker with high cholesterol values.

The pathological post-exercise S-T depressions in diabetics may be caused by many factors, e.g changes in the autonomic nervous system myocardial metabolism or the increased arterial blood pressure. However the high incidence of clinical and/or ECG signs of myocardial infarction in diabetics with pathological post-exercise S-T depression obviously indicates that these ECG changes are due to coronary heart disease. A contribution from the other factors mentioned cannot be excluded however

This follow-up study has shown that a persisting normal exercise ECG or an improvement from an initial abnormal ECG are seen predominantly in diabetics without various other risk factors In the group of subjects who still have, or have developed pathological post-exercise ECG or have sustained a myocardial infarction there is a higher frequency of various risk factors for coronary heart disease. Of these smoking appears to increase the risk of cardiovascular complications considerably It therefore seems advisable to encourage diabetics in particular to stop smoking

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16 36) The high percentages of pathological post-exercise S-T changes in diabetics in 1963 (27%) and in 1972 (50%) are in good agreement with the numerous reports of high incidence of coronary heart disease in diabetics (11 19 28 33 41)

When evaluating the difference in S-T reaction between controls and diabetics it should be remembered that the controls were driven to a higher exercise intensity After equal exercise loads the differences between controls and diabetics would probably be even more striking

However in a computer study Campbell et al (13) compared young controls with age matched diabetics and found significantly less S-T depression during moderate exercise and slower return of S T segment to the resting level after exercise in the diabetics. Consequently the T wave depression at the end of exercise was significantly less in the diabetics but during the recovery period the T wave tended to remain depressed in the diabetics in contrast to the rapid recovery of the T wave height in controls often to a level exceeding that at rest At first sight these findings seem to disagree with the present observations and those of Karlefors (26) We find more numerous and more advanced S-T depressions in diabetics both immediately and 4 minutes after exercise. Both in controls and in diabetics there is a considerable rise in T amplitudes immediately after exercise but 4 minutes after exercise T amplitudes are considerably lower than the resting values in both groups The difference in results may depend upon the higher age of our patients and the heavier work load used

In order to study the influence of smoking on the cardiovascular system the individuals have been divided according to smoking habits It was noted that the non smoking diabetics weigh more and have higher triglyceride values It has been discussed before by the author that this may be due partly to dietary problems (34)

Most cases of pathological S-T changes after exercise are found among the smokers, especially in the diabetics It is also seen that most of the patients in which pathological S-T changes developed since 1963 were smokers. This may be due to a relation between smoking and atherosclerosis of the coronary arteries as has been discussed in several investigations (7 21 22 30 31 32 43) Ditzel et al (18) have suggested that diabetic angiopathy is caused by defective tissue oxygenation related to the low levels of 2,3-diphosphoglycerate Similarly the works of Astrup (i e 5 6) indicate that the intimal damage (leading to atheromatosis) caused by carbon monoxide may depend upon deficient oxygen delivery These observations provide a tentative explanation for the harmful effect of smoking in diabetics

However several other variables and processes may influence the development of S-T changes during the follow up period e g body weight lipid levels heart rate blood pressure and physical activity For the diabetics the disturbance of carbohydrate metabolism duration of diabetes and other diabetic complications as manifested in the degree of retinopathy and the presence of albuminuria may play a role In agreement with others (4 36) we have found the highest frequency of pathological post-exercise S-T depressions in individuals who already in 1963 had slowly ascending S-T depressions Also individuals with rapidly ascending S T depressions in 1963 now appear to have a higher frequency of pathological post exercise S-T depressions than those with normal S-T in 1963 The difference is not significant but it indicates that also rapidly ascending S-T depressions may have some prognostic value Individuals with pathological S-T changes in 1972 have higher mean age cholesterol triglycerides and systolic blood pressure They are more often smokers and have more often a sedentary occupation The diabetics with pathological S-T changes

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Exercise tests in individuals with borderline glucose tolerance and varying smoking habits

by G PERSSON

from the Departments of Clinical Physiology and Internal Medicine, University of Lund, Lund, and from Dalby Community Health Research Centre Dalby, Sweden

ABSTRACT

One hundred and twenty male subjects with known reduced glucose tolerance for at least 9 years were examined with exercise tests and compared with 44 control subjects.

The borderline subjects had been randomized to 4 different groups. The first group was given no treatment at all, the second one dietary instructions, the third dietary instructions and placebo tablets and the fourth dietary instructions and tolbutamide 500 mg three times daily.

Higher systolic blood pressure was observed in the borderline subjects both at rest and during exercise. The controls managed a higher exercise load than the borderline subjects. Non-smokers of all groups managed a higher load than the smokers.

Four minutes after exercise pathological S-T depressions were found in 30% of the controls, but in 37 to 60% of the borderline subjects. The borderline subjects with pathological post-exercise S-T changes had higher systolic blood pressure and lower maximum load values; these subjects were more often smokers and had more often a sedentary occupation as compared to borderline subjects with normal post-exercise ECG reaction.

During the 9 years of observation 12 borderline subjects had died, 8 of them in coronary heart disease. Besides 6 borderline subjects and 1 control subject had ECG signs of myocardial infarction and 4 borderline subjects had developed intermittent claudication.

Most cases of myocardial infarction, pathological S-T changes and intermittent claudication were found in untreated, diet-treated and placebo-treated borderline groups. No case of myocardial infarction or intermittent claudication has occurred in the tolbutamide-treated borderline group. The frequency of pathological S-T changes after work in this group was close to that of the control group.

INTRODUCTION

The frequent occurrence of decreased glucose tolerance in clinical coronary heart disease reported by i.a. Ostrander (24) has been confirmed by more direct evidence relating abnormal coronary angiographic findings to abnormalities of glucose metabolism (15). Of all patients with evident coronary atherosclerosis, however, only a small fraction have manifest diabetes whereas the frequency of mild glucose *intolerance* may exceed 50% (12, 35). The abnormality of glucose metabolism therefore quite likely precedes the development of clinical arterial disease rather than being a secondary phenomenon to the latter (23, 32). Thus hyperglycemia has been added to the long list of "risk factors" of coronary heart disease (13).

In the Bedford study "borderline diabetic"

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One hundred and twenty male subjects with known reduced glucose tolerance for at least 9 years were examined with exercise tests and compared with 44 control subjects.

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Higher systolic blood pressure was observed in the borderline subjects both at rest and during exercise The controls managed a higher exercise load than the borderline subjects Non-smokers of all groups managed a higher load than the smokers.

Four minutes after exercise pathological S-T depressions were found in 30% of the controls but in 37 to 60% of the borderline subjects The borderline subjects with pathological post-exercise S-T changes had higher systolic blood pressure and lower maximum load values these subjects were more often smokers and had more often a sedentary occupation as compared to borderline subjects with normal post-exercise ECG reaction

During the 9 years of observation 1? borderline subject had died 8 of them in coronary heart disease Besides, 6 borderline subjects and 1 control subject had ECG signs of myocardial infarction and 4 borderline subjects had developed intermittent claudication.

Most cases of myocardial infarction, pathological S-T changes and intermittent claudication were found in untreated diet-treated and placebo-treated borderline groups. No case of myocardial infarction or intermittent claudication has occurred in the tolbutamide treated borderline group The frequency of pathological S-T changes after work in this group was close to that of the control group.

INTRODUCTION

The frequent occurrence of decreased glucose tolerance in clinical coronary heart disease reported by i.a. Ostrander (?4) has been confirmed by more direct evidence relating abnormal coronary angiographic findings to abnormalities of glucose metabolism (15). Of all patients with evident coronary atherosclerosis, however only a small fraction have manifest diabetes whereas the frequency of mild glucose intolerance may exceed 50% (1? 35) The abnormality of glucose metabolism therefore quite likely precedes the development of clinical arterial disease rather than being a secondary phenomenon to the latter (?3 32) Thus hyperglycemia has been added to the long list of risk factors of coronary heart disease (13).

In the Bedford study "borderline diabetic

where to continued tablet treatment and left the study

The material was also grouped according to smoking habits. With a few exceptions the subjects had unchanged smoking habits during the observation period. Five individuals that had stopped smoking were excluded from the study.

Of the original 225 subjects thus 164 remained for the study in 1971–1973.

METHODS

The examinations started at 8 o'clock in the morning. Eating or drinking were not allowed from 10 o'clock p.m. the day before. The individuals were asked not to smoke on the day of examination. Blood samples were collected for analysis of sedimentation rate, haemoglobin, number of red cells, packed red cell volume, glucose, aspartate-aminotransferase, alanine-aminotransferase, cholesterol, triglycerides, potassium, uric acid and creatinine. A urine sample was tested for the presence of glucose or albumin.

The individuals were asked about their smoking habits and divided into non-smokers and smokers.

Each subject was classified into 1 of 4 occupational groups with regard to the physical activity involved. Each subject was also asked for cardiovascular symptoms using a standardized questionnaire (31).

All subjects were carefully examined by the same physician with special reference to the circulatory system. Heart rate and blood pressure were measured in supine position after 15 minutes of rest, during the exercise test and immediately, 4 and 10 minutes after [illegible] as pointed out before (17). [illegible] pressure during exercise was not analysed.

A standard 1? lead ECG (I, II, III, aVR, aVL, aVF, V_1, V_2, V_3, V_4, V_5, V_6) was recorded on a 6-channel ink-jet recorder (Elema-Schönander) before and after exercise. During exercise the indifferent electrode for precordial leads was moved to the forehead and consequently CH leads were recorded.

ECG was recorded at rest both in recumbent position and sitting on the bicycle. The exercise test was done sitting on an electrically braked bicycle ergometer in the same way as described before (17) with the exception that the load was graded in watts (W), 50 watts approximately corresponding to 300 kpm/min. The bicycle ergometer used was calibrated regularly.

Exercise started 2–4 hours after breakfast, at 50 watts with determinations of heart rate, blood pressure, breathing frequency and ECG recording every other minute.

During exercise circulatory steady state was considered to exist if the heart rate on a certain work load increased less than 5 beats/min. between 2 observations with 2 minutes interval. If such a steady state had been obtained, the exercise load was increased by 50 watts and a new steady state was awaited. The exercise continued on the same load until a steady state was reached or work had to be stopped. Exercise was stopped when subjective symptoms (e.g. excessive tiredness and/or pain) or increase in heart rate or blood pressure or the occurrence of ECG abnormalities made a continuation of the exercise test inadvisable.

All ECGs were coded separately by two experienced ECG observers. None of them knew whether the ECG belonged to a control or a borderline subject. If the coding results of the two observers differed, the tracings were discussed so that a unanimous opinion was reached (4). The tracings were classified according to a modified Minnesota code. The S-T depressions were coded as *ischemic* (horizontal or downward sloping), *slowly ascending* or *rapidly ascending* according to criteria given by Blackburn and Punsar et al. (3, 29). In the text and tables ischemic and slowly ascending S-T depressions are mostly grouped together and referred to as "patho-

subjects (defined by glucose tolerance test) showed a prevalence of cardiovascular disease intermediate between that found in normals and diabetics (16 20) This suggests that the occurrence of vascular disease is correlated to the severity of glucose intolerance

An important question is obviously if early diagnosis and treatment of slight or moderate glucose intolerance may prevent or delay the development of true diabetes and/or cardiovascular complications Keen and Jarret have shown that borderline diabetics" treated with diet and tolbutamide have a lower frequency of ECG abnormalities (18 19) On the other hand the U G D P study suggested that control of blood glucose levels with anti-diabetic drugs did in fact accelerate cardiovascular complications of diabetes (34) Similarly Hadden et al. (14) and Boyle et al (7) found in retrospective and prospective studies respectively that maturity-onset diabetics treated with oral anti-diabetic agents developed myocardial infarction more frequently than diabetics treated with diet alone

The main purpose of the present study is to examine the occurrence of electrocardiographic abnormalities during exercise in individuals with borderline glucose tolerance and with different types of treatment including tolbutamide A control group with normal glucose tolerance was also studied.

As preliminary analysis pointed at differences in smoking habits and as smoking may influence on the electrocardiographic findings (1) the material was also divided according to smoking habits

MATERIAL

In a diabetes detection survey in Malmöhus county in 1962 to 1965 32% of the population (228,833 individuals) were screened for glucosuria with Clinistix® after a carbohydrate rich meal (9)

Of 2477 individuals with glucosuria 2180 were further examined with an oral glucose tolerance test during standardized conditions Thirty grams of glucose in 10% solution per m² body surface were given to the individuals Blood glucose was measured before the glucose load and after 15 30 45 60 75 90 120 150 and 180 minutes (22)

Diabetes was diagnosed when all 10 blood glucose values exceeded the normal mean plus 3 standard deviations (S D) Individuals with all values below the mean plus 2 S D were considered normal Individuals not fulfilling these criteria were defined as borderline cases (22) There were found 578 cases with borderline glucose tolerance Some of these were randomly selected and divided into 4 equal groups The first group was not treated at all the second one was given dietary instructions the third dietary instructions and placebo tablets and the fourth dietary instructions and tolbutamide 0.5 gram three times daily These 4 groups are referred to as untreated diet" placebo and tolbutamide" group respectively

At the same time age matched controls with no family history of diabetes and with normal glucose tolerance were selected at random from the entire screened population. Most of the borderline cases and the controls have been followed during 10 years

The classification of the individuals in the present study is thus based on the glucose tolerance tests in 1962–1965 The present study was restricted to men aged 30–67 years From the beginning 177 subjects with borderline glucose tolerance and 46 control subjects were men born in 1901 or later (Table I) Since 1962–1965 12 subjects died Twenty five individuals who had developed diabetic glucose tolerance 1 subject with a diastolic blood pressure above 100 mm Hg and 4 individuals with occlusive arteriosclerosis in the legs and intermittent claudication were excluded In the placebo and tolbutamide groups 5 and 7 subjects respectively did not

adhere to continued tablet treatment and left the study

The material was also grouped according to smoking habits With a few exceptions the subjects had unchanged smoking habits during the observation period Five individuals that had stopped smoking were excluded from the study

Of the original 223 subjects thus 164 remained for the study in 1971–1973

METHODS

The examinations started at 8 o clock in the morning Eating or drinking were not allowed from 10 o clock p m the day before The individuals were asked not to smoke on the day of examination. Blood samples were collected for analysis of sedimentation rate hemoglobin, number of red cells, packed red cell volume glucose aspartate-aminotransferase alanine-aminotransferase cholesterol triglycerides potassium uric acid and creatinine A urine sample was tested for the presence of glucose or albumin.

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All subjects were carefully examined by the same physician with special reference to the circulatory system Heart rate and blood pressure were measured in supine position after 15 minutes of rest during the exercise test and immediately 4 and 10 minutes after it end For reasons pointed out before (17) diastolic pressure during exercise was not analysed

A standard 1 lead ECG (I II III aVR, aVL, aVF V V V_4 V_5 V_6) was recorded on 6-channel ink jet recorder (Elema Schönander) before and after exercise During exercise the indifferent electrode for precordial leads was moved to the forehead, and consequently CH leads were recorded.

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TABLE I P rti ipation of subjec i follow-up study in 1 71 1973

Group	Men born in 1904 1 ter parti ipating in 1962 1965	Subjects excluded from the p esen udy beca e of: Diabetes	Hyper tension	Cl udi cation	Ex smoking	Change f therapy	Death	P ti ipant h f llow-up tu in 1971 1973: To 1 no	Smokers
Borde line groups									
Untreated	61	15	1	2	0	0	2	41	24
Diet	41	4	0	1	2	0	3	32	22
Placebo	38	4	0	1	0	5	8	20	9
Tolbutamide	37	2	0	0	1	7	0	27	14
Control	46	0	0	0	2	0	0	44	22

TABLE II Descripti f the materi l in 1971 1973

Mean values ± S E M

Group	No f objects	Ag (year)	Height (cm)	Weight (kg)	Blood glucose (mMol/l)	Cholesterol (mMol/l)	Triglycerides (mMol/l)
Bo de line groups							
Untreated	41	52 8 1 18	172 1 15	79 3 1 93	4 7 0 12	6 8± 0 15	1 2 ± 0 11
Diet	32	54 8 ± 1 74	171 1 24	74 8 ±2 28	4 9 ± 0 22	6 8± 0 18	1 1 ± 0 15
Placebo	20	58 8 2 28	173 1 48	76 7 2 08	4 4 0 10	6 6± 0 26	0 8 0 07
Tolbutamide	27	55 5 1 81	175 1 10	79 6 2 04	4 8 0 13	6 5± 0 25	0 7 0 06
Controls	44	49 7 ± 1 53	175 ± 0 97	75 2 1 39	4 5 ± 0.09	6 3 0 07	1 1 ± 0 18

TABLE III Occupation

Group	1 Predomi mostly sitting	2 Si ting tanding some walking	3 Walking some handling f mater al	4 Heavy manual work
Borde line groups				
Untre ted	11	9	13	8
Di	5	13	11	3
Placebo	5	6	9	0
Tolbutamide	5	7	11	4
Control	8	18	11	7

gnal S-T changes since Punsar et al. found that these S-T depressions were associated with increased morbidity in onary heart disease (6 29) To obtain a quantitative estimate of T wave changes, amplitude measurements were made of R nd T waves in lead V_6 and the T/R ratio was alculated (17 25)

For the statistical comparison between ontrols and borderline cases Student's t-test and Chi²-test were used Conventional probability levels of significance were applied

RESULTS

Table II describes some characteristics of the groups in 1971–1973 The mean age varied between 50 and 59 years. The mean age in he diet, placebo and tolbutamide groups ere higher than in the control group ($p < 0.05$) There were no important differ ences in height or body weight.

Table III describes the occupations of the subjects There was no significant difference between the groups The diet and placebo groups and the controls had a slight preponderance of occupations involving lighter work.

The fasting blood glucose alues were with n normal range and did not differ significanth between the groups However the resuh of glucose tolerance tests in the borderline groups deviated significantly from those of the ontrols (8) The cholesterol alues n the unt eated and diet groups were higher than in th control ($p < 0.05$) and trigh erides were l wer n the tolbutamide group th n in the other groups ($p < 0.05$) ex ept for the placebo group Mean values for holester l and triglycerides ex eeded however in n group the normal range

The frequenc of mokers varied between 45 nd 69% being highest in the diet group Ther were n significant differences between mokers and non smokers as regards the variables in Tables II and III

The untreated group had higher heart rate at rest than the controls and the placebo group ($p < 0.01$) (Table IV) Systolic blood pressure at rest was higher in borderline groups than in controls ($p < 0.01$) except for the tolbutamide group (Table V) Diastolic pressure in the untreated group was higher than in the tolbutamide group and the controls ($p < 0.05$) (26) In all borderline groups pulse pressure was higher than in the controls ($p < 0.01$) (26)

During exercise in all groups heart rate at steady state rose with about 20–30 beats/min for each load At 150 watts, at maximum load and 4 minutes after work there was no significant difference in heart rate neither between controls and borderline groups nor between the different borderline groups, except for the untreated group which 4 minutes after work had higher heart rate than the placebo group ($p < 0.05$) (Table IV)

Systolic blood pressure rose with about 20 mm Hg for each load both in the control group and in the borderline groups (Table V). Only at work loads of 50 and 100 watts was the difference in systolic blood pressure between the diet and placebo groups and the controls significant ($p < 0.05$) The differ ences in systolic pressure were smaller at maximum load. In all groups the systolic blood pressure 4 minutes after work was nearly the same as that at rest.

The controls performed a higher maximum work load than the borderline groups ($p < 0.05$) except for the untreated ones (Table IV)

Among smokers the untreated group had a higher heart rate than controls and placebo group ($p < 0.05$). Among non-smokers, there was no significant difference between the groups (Table VI). The systolic blood pressure at rest showed the same differences between the groups in smokers and non-smokers as in the total material (Table VII). In no group were there any significant differ ences between the smokers and non-smokers

TABLE IV Heart rate at rest during and after exercise. Maximum load

Mean values ± S E M

Group	Heart rate (beats/min) At rest	50 W steady state	100 W steady state	150 W steady state	At maximum load	4 min after exercise	Maximum load (W)
Borderline groups							
Untreated	74 1 7	105 2 7	128 2 6	152 3 7	163 3 9	100 ± 3 4	170 7 4
Diet	71 ± 3 8	102 2 1	130 3 5	160 6 1	162 3 0	97 2 3	152 6.3
Placebo	65 2 3	94 2 2	122 2 5	153 5 3	158 4 6	91 3 0	158 7 3
Tolbutamide	70 1 9	102 2 4	130 ± 3 6	156 5 8	159 ± 3 7	93 3 0	156 4 9
Control	67 1 7	97 3 0	123 2 7	151 3 2	167 2 4	97 3 2	180 5 9

TABLE V Systolic blood pressure at rest during and after exercise

Mean values ± S E M

Group	Systolic pressure (mm Hg) At rest	50 W steady state	100 steady state	150 steady state	At maximum load	4 min after exercise
Borderline groups						
Untreated	145 3 5	157 3 2	178 3 7	199 4 6	208 3 7	145 3 6
Diet	145 2 8	162 3 1	186 4 0	202 3 6	212 3 8	149 2 8
Placebo	146 4 3	164 5 3	190 ± 5 4	194 4 5	218 ± 4 3	149 4 5
Tolbutamide	139 2 4	157 ± 3 2	179 4 0	199 7 4	205 3 9	140 ± 2 2
Controls	138 1 9	150 ± 2 4	171 ± 2 5	193 3 7	202 2	136 ± 2 2

TABLE VI Heart rate at rest during and after exercise and maximum load, in relation to smoking habits

Mean values ± S E M

Group	No of subjects	Heart rate (beats/min) At rest	50 W steady state	100 W steady state	150 W steady state	At maximum load	4 min after exercise	Maximum load (W)
Smokers								
Borderline groups								
Untreated	24	74±2 0	107 3 7	129 3 6	147 4 3	158 3 3	2	154 23
Diet	22	71±2 6	101 ± 2 6	128 4 3	162 6 1	15 8	96 3	148 25
Placebo	9	63 3 1	95 4 0	123 ± 4 0	167 4	164 6 9	4 4	156 16
Tolbutamide	14	71 3 0	102 3 4	128 4	145 ± 6 1	155	2	15 52
Controls	22	67±2 3	99 3	127 3 9	136 ± 4 9	16 3 8	2 7	8 ± 23
Non-smokers								
Borderline groups								
Untreated	17	75±3 0	102 1 9	127 3 7	155 5 8	170 5 0	4	168
Diet	10	78 2 9	104 3 5	13 5 6	155 ± 5 4	168	99	163
Placebo	12	66 3 5	94 2 4	122 3 3	143 3 3	153 6	98 4 3	15 13
Tolbutamide	13	69-2 5	102 ± 3 5	132 5 5	165 5	1 5	94 ±	6 63
Control	33	68 2 1	95 2 8	119 3 7	14 4 8	1 8 3 8	98 3	19 63

TABLE VII. Systolic blood pressure at rest, during and after exercise in relation to smoking habits.

Mean values ± S.E.

Group	No. of subjects	Systolic pressure (mm Hg) At rest	50 W steady state	100 W steady state	150 W steady state	At maximum load	4 min after exercise
Smokers							
Borderline groups							
Untreated			18 ± 9	1 ± 5	260	21 .2	7 ± 8
Diet	22	146 3	62	8 5 1	204 4	1	1
Acarbose		1 6 3	61	181 8	290 ± 8	205 ± .5	148 ±
Tolbutamide	1	1 1 ± 3	1 1	1 7 ± 5	18 9	207	3
Controls	22	38 ± 3 6	158	1 3 7	9 ± 6 4	208 .3	13
Non-smokers							
Borderline groups							
Untreated		± 3 2	156 ± 8	75	9	84 5 1	1 2 4 1
Diet	1	2	4 3	1	1 4	21	158 ± 3 8
Acarbose		1	157	3 ±	1 3	213 5	58 1
Tolbutamide		134 ± 6	1 ± 4	1 ±	± 5	203	137 ± 2 3
Controls	3	36 3 7	9 3 1	73 ± 3	±	20 2	139 ± 8

TABLE VIII. ST depression at rest and 4 min after exercise

Group	No. subjects	ST ischaemic depression	ST slowly ascending	Rapidly ascending	No depression	Pathological	ST 4 min after exercise: ischaemic depression	Slowly ascending	Rapidly ascending	No depression	Pathological (%)
Borderline groups											
Untreated					35	12	8	12		1	
Diet	33				26		8			1	37
Acarbose					5			9			9
Tolbutamide						5					37
Controls		1							9	22	35

TABLE IV Heart te t re t du ing nd fte xerci Maximum load

Mea lues ± S E M

Group	He rt te (be t /min) At rest	50W steady tat	100W t ady ta	150 W teady tat	At maximum load	4 i (t rcise	Ma imum load (w)
Borde line groups							
Untreated	74 1 7	105 2 7	128 2 6	152 3 7	163 2 9	100 2 4	170 7 0
Diet	71 2 0	102 2 1	130 3 5	160 6 1	162 3 8	97 2 3	152 6 9
Placebo	65 2 3	94 2 2	122 2 5	153 5 3	158 4 6	91 3 0	158 7 5
Tolbutamide	70 1 9	102 2 4	130 3 6	156 5 8	159 3 7	93 3 0	156 4 9
Control	67 1 7	97 2 0	123 2 7	151 3 2	167 2 4	97 2 3	188 5 8

TABLE V Systolic blood pressu t r at du ing nd ft exe i

Mean value ± S E M

Group	Sys li pres re (mm Hg) At res	50 W teady tat	100 W teady ta	150 W eady t t	At maximum load	4 mi f xe ise
Borde line groups						
Untreated	145 3 5	157 3 2	178 3 7	199 4 6	208 3 7	145 3 6
Diet	145 2 8	162 ± 3 1	186 4 0	202 3 6	212 3 8	149 2 8
Placebo	146 ± 4 3	164 5 3	198 5 4	194 4 5	210 4 3	149 ± 4 5
Tolbutamide	139 2 4	157 3 2	179 4 0	199 7 4	205 3 9	140 2 2
Control	136 ± 1 9	150 2 4	171 2 5	193 ± 3 7	202 2 9	136 2 2

TABLE VI Heart rate at res during nd fter ex rcise and maximum load in rel on smoking hab ts

Mean values ± S.E.M.

Group	No f subjects	Heart ra (beats/min) res	50 W steady tate	100 W eady sta	150 W steady te	At maximum load	4 mi f re se	axirum load (W)
Smokers								
Borderline groups								
Untrea ed	24	74 2 8	107 3 7	129 3 6	147 4 3	158 3 3	98 2	154 2.9
Di	22	71 2 6	101 2 6	126 4 3	162 6 1	159 3 8	96 3	148 7.8
Placebo	9	63–3 1	95 4 0	123 4 0	16 4 8	16 6	93 4	156 10.8
Tolbutamide	14	71 3 0	202 3 4	128 4 9	145	55	2 4 1	150 5.2
Con rols	22	67 2 3	99 2 7	127 3 9	156 4 9	167 3 8	9 ± 2	170 7.3
Non-smok rs								
Borde line groups								
Un re ted	17	75–3 0	102 3 9	12 3 7	155 5	170 5 8	101 5	1 2 10.8
Di	10	70–2 9	106 3 5	13 ± 5 6	155 5 4	1 8 3	99 3 1	160 16.3
Placebo	11	66 3 5	9 2 4	122 3 3	1 5 5 3	153 6 0	90 4 2	159 11.3
T lbutamide	13	69–2 5	102 ± 3 5	132 5 5	165 7 5	16 5 6	9 ± 4	162 8.3
Con rol	22	68 2 1	95 ± 2 8	119 3 7	1 6 4 8	1 8 3	98 3 5	191 8.5

TABLE VII [illegible] blood pressure [illegible] rest, during and [illegible] exercise [illegible] to [illegible] habits.

Mean values ± S.E.M

Group	No. of subject	[illegible] (mm Hg) At rest	50 W steady st.	100 W steady state	150 W steady state	At maximum load	4 min after exercise
[illegible]							
Border line groups							
[illegible]		147 5 4	155 ±	180 ± 5 4	200 9 7	2 0 5 2	47 ± 5 4
[illegible]	2	144 9	142	18 1	20 ±	2 0 4	149 ± 3 7
[illegible]	9	1 4 ±	141 7 0	183 ± .8	200 ± 5 8	203 ± 4 5	1 8 ± 7
[illegible]	4	141 ± 3	1 ±	8 ±	21 ± 2	207 3 3	143 3 9
Controls	22	135 ±	50	170 7	19 ± 4	200 ± 3	133 2
[illegible]							
Border line groups							
[illegible]		± 3 2	156 ±	1 3 5 0	90	204 5	14 1
[illegible]	6	44 3	3 ±	9		21	158 ± 3 8
[illegible]	1		7	5 ± 8	51 3	2 5 5 0	50 1
[illegible]		36	5 ±	77 ± 8	1 1 5	202 ± 4	137 ± 2 3
Control	13	16	09	73 3	193 ±	20 1	139 ± 3.0

TABLE VIII [illegible]

Group	No. subjects	[illegible] depression	Slowly ascending	Rapidly ascending	No depression	[illegible] (%)	[illegible] depression	Slowly ascending	Rapidly ascending	No depression	[illegible] (%)
Border [illegible] groups											
[illegible]						13					
[illegible]					26					3	
[illegible]	20				1			1			40
[illegible]						1			2	5	37
[illegible]									9		30

TABLE IV Heart te t re t during nd f rercise Maximum load
Mean value ± S E.M

Group	Heart te (be ts/mi) At re t		50W steady tate		100W t ady tat		150 W t ady tat		At maximum load		4 i ft rercise		Ma imum load (W)	
Borde line groups														
U tre ted	74	1 7	105 ±	2 7	128	2 6	152	3 7	161	2 9	100	2 4	170	7 8
Diet	71	2 0	102	2 1	130	3 5	160	6 1	162	3 0	97	2 3	157	6 9
Placebo	65	2 3	94	2 2	122	2 5	153	5 3	158	4 6	91	3 8	158	7 5
Tolbutamide	70	1 9	102	2 4	130	3 6	156	5 8	159 ±	3 7	93	3 0	156	4 9
Control	67	1 7	97	2 0	123	2 7	151	3 2	167	2 4	97	2 2	180	5 8

TABLE V Systolic blood pressure at rest du ing nd ft exe i
Mea values S E M

Group	Systoli pressure (mm Hg) At re		50 teady		100 W teady ta		150 teady tat		A maximum load		4 mi f exercise	
Borderline groups												
Untreated	145	3 5	157	3 2	178	3 7	199	4 6	208	3 7	145	3 6
Di t	145	2 8	162	3 1	186 ±	4 0	202	3 6	212	3 8	149	2 8
Placebo	146	4 3	164	5 3	190 ±	5 4	194	4 5	218	4 3	149	4 5
Tolbutamide	139	2 4	157	3 2	179	4 0	199 ±	7 4	205 ±	3 9	140 ±	2 2
Control	136 ±	1 9	158 ±	2 4	171 ±	2 5	193 ±	3 7	202 ±	2 9	134 ±	2 2

TABLE VI Heart ra t res during and af exerc se and maximum load in rel ion to smoking habits
Mean values S E M

Group	No f subjects	Heart r te (beats/min) A rest		50 ady st		100 W st ady state		150 W t ady ate		At ma imum load		4 i ft rercise		aximum load (W)	
Smok rs																
Borde line groups																
Untrea ed	24	74±2	8	107	3 7	128	3 6	147	4 3	158	3 3	98	2	194	7.9
Diet	22	71	2 6	101	2 6	126	3	142	6 1	159	3 8	96	3 0	148 ±	1.0
lacebo	9	63-3	1	95	4 0	123	4 8	167	4 8	164 ±	4	93		156	10.8
Tolbutamide	14	71±3	0	102	3 4	128	4 9	145 ±	6 1	1 5 ±	4 7	92	4 1	1	5.2
Control	22	67±2	3	99	2 7	127	3 9	15	4 9	167	3 8	97	2	17	[illegible]
Non-smok rs																
Borde line groups																
Un reated	17	75-3	0	102	3 9	127	3 7	155	5 4	1 8	5 8	01	5	192	10.8
Di	18	70±2	9	106	3 5	127 ±	5 6	155	5 4	168	3	9	3 1	168	6.3
Placebo	11	66	3 5	94	2 4	122	3 3	1 5	5 3	153	6 8	0	4 2	159	11.3
Tolbutamide	13	69-2	5	102 ±	3 5	132	5 5	145	7 5	164	5 6	94 ±		162	8.3
Con rol	22	68	2 1	95	2 8	119	3 7	146	4 0	168	3 8	8	3 5	1 1 ±	8.5

TABLE VII [illegible] blood pressure at rest, during and after exercise [illegible] smoking habits

Mean values ± S.E.M.

Group	No. of subjects	At rest	50 W steady state	100 W steady state	150 W steady state	At maximum load	min after exercise
Smokers							
Borderline groups							
Untreated		1 7 5 6	158 4 9	180 5	209 9	21 3	1
Diet	22	±	142 3	1 3	204 4	218	149 ± 3 7
[illegible]	9	6 3	1 1 ±	3 ±	205 ± 6	1 5 ±	14 7 1
Tolbutamide	1	± 3 6	1 ±	4 ±	10 ± 13 9	207 ± 3	1 1 3
Controls	33	136 ± 2	58 ± 3	78 7	1 3	209 3	133
Non-smokers							
Borderline groups							
Untreated		±	154 ± 3	175 ±	198	204 ± 5 1	142 1
Diet	1	44 ±	1 3		197	214 ±	150 3 6
[illegible]		±	±	5 ±	1 1 ± 6 3	215 ± 5 6	150 ± 6 1
Tolbutamide		36 ± 1	151 ±	77 ± 6	1 ± 7 5	38	3
Controls	33	136	1	173 ± 3	1 3 ±	38 1	139 ±

TABLE VIII [illegible] at rest and 4 minutes after exercise

Group	No. subjects	S-T at rest: Ischaemic depression	Slowly ascending	Rapidly ascending	No depression	Pathological (%)	S-T 4 min. after exercise: Ischaemic depression	Slowly ascending	Rapidly ascending	No depression	Pathological (%)
Borderline groups											
Untreated				1	1	13		13			
Diet					3	4			7	3	
[illegible]	30	3			15	15	2			7	9
Tolbutamide		1		6	3	15	3				37
Controls		1	2		40					3	30

as regards heart rate and systolic pressure during exercise (Tables VI and VII) *Only in* the untreated group was the maximum load significantly higher in non smokers than in smokers ($p < 0.01$) Among *smokers* controls had higher maximum loads *than* diet and tolbutamide groups ($p < 0.05$) In non-smokers controls and the untreated group had significantly higher *maximum loads than* the placebo and tolbutamide groups (Table VI)

Three out of 44 controls (7%) and 17 of 120 borderline cases (14%) had pathological S-T changes at rest. The frequency of S-T abnormalities varied in the different borderline groups between 12 and 16% (Table VIII)

In borderline groups 11 *of* 69 *smokers* (16%) and 6 of 51 *non smokers* (12%) had ischemic *or* slowly ascending S-T segments at rest *In controls 2* of 22 *smokers and 1 of 22 non-smokers* had pathological S-T changes at *rest* (Table IX) The small number of patients with *ECG* abnormalities at *rest* obviously invalidates the analysis.

S-T changes especially during but also immediately after exercise may be difficult to evaluate due to technical problems (17) Instead special attention was given to S-T *changes 4 minutes after work. Lead* V_5 *was* used for analysis

Pathological S-T changes 4 minutes after exercise were found in 13 of 44 controls (30%) and in 54 of 120 borderline cases (45%) the differences being significant ($p < 0.05$) (Table VIII) The frequency of S-T abnormalities in borderline cases was higher in the untreated and in the placebo groups 49 and 60% respectively than in the diet and tolbutamide groups 37% in each

In controls pathological post-exercise S-T changes were found in 27% of the smokers and in 32% of the non smokers (*Table IX*) By contrast in the borderline groups more cases of pathological post-exercise changes were seen in the *smokers* (51%) *than* in the *non smokers* (37%) However if o[illegible] *more severe ischemic type of S-T chang[illegible] considered (Table IX)* the differenc[illegible] *tween* smokers and non smokers woul[illegible] significant. Thus there were 23% [illegible] S-T segments among the smokers but [illegible] 10% among the non smokers ($p < 0.05$).

Among the borderline groups the hi[illegible] *frequency of pathological post-exercise* changes was seen in the smoking pl[illegible] group (67%) On the contrary the [illegible] frequency of pathological post-exerc[illegible] changes was seen in non smoking diet tolbutamide groups 20 and 23% re[illegible]*tively*

In order to correlate the ECG reaction[illegible] minutes after work with different d[illegible] data, *controls* and borderline cases have separated in those having normal pathological ECG reactions [illegible] (Table X) *In controls* there were no sign[illegible]cant differences between individuals w[illegible] normal and those with pathological EC[illegible] *reactions as regards age* weight, lipids bl[illegible] glucose values blood pressure max[illegible] load smoking habits and occupation In t[illegible] combined borderline groups those [illegible] pathological post-exercise S-T depressi[illegible] had higher systolic and diastolic blood *pressures* and *lower* maximum load va[illegible] ($p < 0.01$)

It should also be mentioned that th[illegible] percentage of smokers among border[illegible] cases with pathological ECG reactions [illegible] 65% against 52% of smokers amon[illegible] borderline cases with normal ECG reacti[illegible] after work.

In addition it was apparent that borderline cases with pathological post-exercise S-T changes *tended to have a higher frequency* of sedentary occupations (31/54 57%) than those borderline cases with normal post exercise ECG (30/66 45%) The diff[illegible] in smoking and occupational activities not significant

Measurements of T wave amplitude and

Table IX. ST-segment depression at rest and 4 minutes after exercise in relation to [illegible]

Group	No subjects	ST at rest: Ischaemic depression	Slowly ascending	Rapidly ascending	No depression	Pathological (%)	ST 4 min after exercise: Ischaemic depression	Slowly ascending	Rapidly ascending	No depression	Pathological (%)
Men											
Borderline groups											
Untreated	3	8	2	1	2	8	7	5	3	10	5
Diet	22	2				3	6				45
Placebo	9		1			23	1	5	1	2	67
Tolbutamide	1		1		1	2		5		6	10
Controls	33		1		30	5				13	3
Women											
Borderline groups											
Untreated					1	8	1	7			7
Diet	1						2				20
Placebo	1					9					3
Tolbutamide					12						
Controls	33		1		28					6	33

Table X. Clinical data in relation to ST-segment 4 minutes after exercise

Mean values ± [illegible]

Group	ST-segment after exercise	No of subjects	Age (years)	Blood glucose (mmol/l)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Relative weight (%)	Consumption of tobacco (g/day)	Smoking habits: Non-smokers	Smokers
Borderline groups	Normal		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	32	34
	Pathological		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	19	35
Controls	Normal	11	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]	15	16
	Pathological		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

Group	ST-segment after exercise	No of subjects	Occupation: 1	2	3	4
Borderline groups	Normal	64	10	20		
	Pathological	54	6	1		
Control	Normal	11		12	6	
	Pathological					6

[illegible]

as regards heart rate and systolic pressure during exercise (Tables VI and VII). Only in the untreated group was the maximum load significantly higher in non-smokers than in smokers ($p<0.01$). Among smokers controls had higher maximum loads than diet and tolbutamide groups ($p<0.05$). In non-smokers controls and the untreated group had significantly higher maximum loads than the placebo and tolbutamide groups (Table VI).

Three out of 44 controls (7%) and 17 of 120 borderline cases (14%) had pathological S-T changes at rest. The frequency of S-T abnormalities varied in the different borderline groups between 12 and 16% (Table VIII).

In borderline groups 11 of 69 smokers (16%) and 6 of 51 non-smokers (12%) had ischemic or slowly ascending S-T segments at rest. In controls 2 of 22 smokers and 1 of 22 non-smokers had pathological S-T changes at rest (Table IX). The small number of patients with ECG abnormalities at rest obviously invalidates the analysis.

S-T changes, especially during but also immediately after exercise, may be difficult to evaluate due to technical problems (17). Instead special attention was given to S-T changes 4 minutes after work. Lead V_4 was used for analysis.

Pathological S-T changes 4 minutes after exercise were found in 13 of 44 controls (30%) and in 54 of 120 borderline cases (45%), the differences being significant ($p<0.05$) (Table VIII). The frequency of S-T abnormalities in borderline cases was higher in the untreated and in the placebo groups, 49 and 60% respectively, than in the diet and tolbutamide groups, 37% in each.

In controls pathological post-exercise S-T changes were found in 27% of the smokers and in 32% of the non-smokers (Table IX). By contrast, in the borderline groups more cases of pathological post-exercise changes were seen in the smokers (51%) than in the non-smokers (37%). However, if only the more severe ischemic type of S-T change was considered (Table IX), the difference between smokers and non-smokers would be significant. Thus there were 23% ischemic S-T segments among the smokers but only 10% among the non-smokers ($p<0.05$).

Among the borderline groups the highest frequency of pathological post-exercise S-T changes was seen in the smoking placebo group (67%). On the contrary, the lowest frequency of pathological post-exercise S-T changes was seen in non-smoking diet and tolbutamide groups, 20 and 25%, respectively.

In order to correlate the ECG reactions 4 minutes after work with different clinical data controls and borderline cases have been separated in those having normal and pathological ECG reactions respectively (Table X). In controls there were no significant differences between individuals with normal and those with pathological ECG reactions as regards age, weight, lipids, blood glucose values, blood pressure, maximum load, smoking habits and occupation. In the combined borderline groups those with pathological post-exercise S-T depressions had higher systolic and diastolic blood pressures and lower maximum load values ($p<0.01$).

It should also be mentioned that the percentage of smokers among borderline cases with pathological ECG reactions was 65% against 52% of smokers among borderline cases with normal ECG reactions after work.

In addition it was apparent that borderline cases with pathological post-exercise S-T changes tended to have a higher frequency of sedentary occupations (31/54, 57%) than those borderline cases with normal post-exercise ECG (30/66, 45%). The differences in smoking and occupational activities were not significant.

Measurements of T wave amplitude and

tions and development of diabetes will be presented in detail in a separate study involving a larger series of patients (27)

The higher systolic pressure in borderline groups both at rest and during exercise, may be due to more advanced arteriosclerosis and ageing diminished elasticity of arterial walls and/or other factors discussed in a previous study (25)

The higher maximum load values in controls might be due to the lower mean age in this group and thereby a better physical fitness than in the borderline groups. However this is certainly not the whole truth because the difference in maximum load values between controls and most of the borderline groups is too large to be explained by 5-6 years of age.

In addition when the material is divided according to smoking habits, there are in non-smokers, in spite of similar mean ages significant differences of maximum load values between controls and some of the borderline groups. It is also apparent that non-smokers in all groups have better physical fitness than the smokers. When comparing controls and the untreated borderline group it is also evident that smoking in combination with reduced glucose tolerance decreases maximum load considerably more than smoking only

The 30% pathological post-exercise S-T depressions observed in controls correspond rather well with previous studies in normal subjects (2 10 25) The higher percentages of pathological post-exercise S-T changes in the borderline groups 37 to 60% are in good agreement with numerous reports asserting that hyperglycemia is associated with higher risk for developing coronary heart disease (8 11 13 33) The difference between controls and borderline subjects would of course be even more apparent if not so many borderline subjects had died or been excluded because of arteriosclerotic disease (Table XI).

The relation between smoking and atherosclerosis of the coronary arteries has been discussed in several investigations (1 3 21 30) Also in this study the borderline cases with pathological post-exercise S-T changes have a higher frequency of smokers

In the controls however there is no correlation between smoking and occurrence of pathological post-exercise S-T changes, and in the borderline groups the differences are not so large as in the study of diabetics (25) The 10 years higher mean age in the present material may explain the divergent results. It has been found that the higher incidence of myocardial infarction in smokers tends to disappear in higher age groups (36) It should also be remembered that smoking habits co-vary with other things such as hereditary psycho-social factors, alcohol intake, coffee drinking etc. A detailed analysis of these factors or of physical spare time activity was not attempted in this study However we found that practically all individuals were coffee drinkers and that none had apparent alcoholic or social problems.

In this material we found significantly higher blood pressure values and significantly lower maximum load values in pathological" borderline subjects compared to borderline subjects with normal post-exercise S-T reactions

Just as Wilhelmsen et al. (37) we found that individuals with sedentary occupation tended to have a higher frequency of pathological post-exercise ECG changes.

The cholesterol triglycerides and fasting blood glucose values varied in the different groups but no correlation between the levels of serum lipids and blood glucose and ECG reactions could be detected in this material.

Tolbutamide treatment has been much debated during the last years because of reports of higher frequency of arterial complications in individuals given this drug (7 14 34) The present study however supports observations (18 19) of a protective

the ratio of T to R confirmed the results obtained by S-T coding subjects having pathological S-T depressions showing significantly lower values.

Arteriosclerotic complications in borderline subjects (Table XI)

During the observation period from 1962 to 1972 12 individuals died 8 of them in coronary heart disease according to the death certificates Of the 8 coronary deaths 1 occurred in the untreated group 1 in the diet group and 6 in the placebo group None has died in the tolbutamide group Besides 4 individuals developed intermittent claudication 2 of them belonging to the untreated group 1 to the diet group and 1 to the placebo group Electrocardiographic signs of myocardial infarctions (abnormal R progression or QS-complexes) were found in 1 control subject in 8 cases of the untreated group in 2 of the diet group and in 5 of the placebo group

Thus the total incidence of arteriosclerotic complications in subjects with borderline glucose tolerance was 27 of 160 cases (17%) No case of vascular complication has occurred in the tolbutamide group The incidence of complications in this group was significantly different from that in the untreated and placebo groups ($p<0.05$ and $p<0.001$ respectively) but not from that in the diet and control groups On the contrary the placebo group had a significantly higher frequency of vascular complications than all the other groups except the untreated group The incidence of complications in the untreated group was higher than in the control group ($p<0.05$)

TABLE XI [illegible]

Group	No of subjects Total no	No of exclusions	Peripheral arterial disease	Myocardial infarction ECG signs	Dead in coronary heart disease
Borderline groups					
Untreated	61	59	2	8	1
Diet	41	40	1	2	1
Placebo	38	31	1	5	6
Tolbutamide	37	30	0	0	0
Control	46	46	0	1	0

Subjects with [illegible] and subjects dead of other causes excluded (cf Table II)

Development of diabetes (Table I)

During 9 years 25 subjects developed diabetes 15 in the untreated group 4 in the diet group 4 in the placebo group 2 in the tolbutamide group and none among the controls The frequency of diabetes was significantly higher in the untreated borderline subjects as compared to both the controls ($p<0.001$) and to the "treated borderline subjects regarded as one group ($p<0.05$)

DISCUSSION

In the present study subjects with borderline glucose tolerance were compared with individuals with normal glucose tolerance in an exercise test. The primary aim was to analyse the ECG reactions in relation to reduced glucose tolerance and different types of treatment The individuals have been followed since 1962–1965 when they were selected from a diabetes detection survey

The mortality cardiovascular complica

tions and development of diabetes will be presented in detail in a separate study involving a larger series of patients (27)

The higher systolic pressure in borderline groups both at rest and during exercise may be due to more advanced arteriosclerosis and aging diminished elasticity of arterial walls and/or other factors discussed in a previous study (25)

The higher maximum load values in controls might be due to the lower mean age in this group and thereby a better physical fitness than in the borderline groups. However this is certainly not the whole truth because the difference in maximum load values between controls and most of the borderline groups is too large to be explained by 5–6 years of age

In addition, when the material is divided according to smoking habits, there are in non-smokers in spite of similar mean ages, significant differences of maximum load values between controls and some of the borderline groups. It is also apparent that non-smokers in all groups have better physical fitness than the smokers When comparing controls and the untreated borderline group it is also evident that smoking in combination with reduced glucose tolerance decreases maximum load considerably more than smoking only

The 30% pathological post-exercise S-T depressions observed in controls correspond rather well with previous studies in normal subjects (9 10 25) The higher percentages of pathological post-exercise S-T changes in the borderline groups 37 to 80% are in good agreement with numerous reports asserting that hyperglycemia is associated with higher risk for developing coronary heart disease (8 11 13 33) The difference between controls and borderline subjects would of course be even more apparent, if not so many borderline subjects had died or been excluded because of arteriosclerotic disease (Table XI)

The relation between smoking and atherosclerosis of the coronary arteries has been discussed in several investigations (1 3 21 30) Also in this study the borderline cases with pathological post-exercise S-T changes have a higher frequency of smokers

In the controls however there is no correlation between smoking and occurrence of pathological post-exercise S-T changes and in the borderline groups the differences are not so large as in the study of diabetics (25) The 10 years higher mean age in the present material may explain the divergent results It has been found that the higher incidence of myocardial infarction in smokers tends to disappear in higher age groups (36) It should also be remembered that smoking habits co-vary with other things such as hereditary psycho-social factors alcohol intake, coffee drinking etc. A detailed analysis of these factors or of physical spare time activity was not attempted in this study However we found that practically all individuals were coffee drinkers and that none had apparent alcoholic or social problems.

In this material we found significantly higher blood pressure values and significantly lower maximum load values in "pathological" borderline subjects compared to borderline subjects with normal post-exercise S-T reactions.

Just as Wilhelmsen et al. (37) we found that individuals with sedentary occupation tended to have a higher frequency of pathological post-exercise ECG changes.

The cholesterol, triglycerides and fasting blood glucose values varied in the different groups but no correlation between the levels of serum lipids and blood glucose and ECG reactions could be detected in this material.

Tolbutamide treatment has been much debated during the last years because of reports of higher frequency of arterial complications in individuals given this drug (7 14 34) The present study however supports observations (18 19) of a protective

effect of treatment with tolbutamide since no coronary death myocardial infarction or intermittent claudication was recorded in the tolbutamide group The frequency of pathological S-T changes after work in the tolbutamide group was also close to that of the controls

The frequency of vascular complications (coronary death myocardial infarction and intermittent claudication) was significantly higher in the placebo group than in the diet and tolbutamide groups This was quite unexpected particularly the difference to the diet group since the dietary instructions given to the placebo group were the same as those given to the diet group One possible explanation is that those individuals who were given tablets (placebo or tolbutamide) felt safety" and therefore did not follow the dietary instructions as carefully as those on diet "only If that is the case the lower number of arteriosclerotic complications and *pathological S-T depressions in the tolbutamide group could be due to lower blood glucose levels during the day because of the* active" drug treatment which thus compensated for the dietary carelessness" This explanation may also account for the (statistically not significant) differences between the placebo group and the diet and tolbutamide groups as regards the frequency of pathological S-T changes after work. However other factors may also play a role

The highest frequencies of pathological post-exercise S-T changes were found in the untreated and placebo groups *this tallies* well with the higher incidence of clinical cardiovascular *complications* in these two groups

The present study confirms earlier observations of hyperglycemia as an important risk factor for coronary heart disease. Treatment with tolbutamide is likely to afford protection Furthermore as has been noted in the parallel studies (25 26) smoking in combination with reduced glucose tolerance appears to increase the risk of cardiovascular complications

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effect of treatment with tolbutamide, since no coronary death myocardial infarction or intermittent claudication was recorded in the tolbutamide group The frequency of pathological S-T changes after work in the tolbutamide group was also close to that of the controls

The frequency of vascular complications (coronary death myocardial infarction and intermittent claudication) was significantly higher in the placebo group than in the diet and tolbutamide groups This was quite unexpected particularly the difference to the diet group since the dietary instructions given to the placebo group were the same as those given to the diet group One possible explanation is that those individuals who were given tablets (placebo or tolbutamide) felt safety" and therefore did not follow the dietary instructions as carefully as those on diet "only If that is the case the lower number of arteriosclerotic complications and pathological S-T depressions in the tolbutamide group could be due to lower blood glucose levels during the day because of the active" drug treatment which thus compensated for the dietary carelessness" This explanation may also account for the (statistically not significant) differences between the placebo group and the diet and tolbutamide groups as regards the frequency of pathological S-T changes after work. However other factors may also play a role.

The highest frequencies of pathological post-exercise S-T changes were found in the untreated and placebo groups this tallies well with the higher incidence of clinical cardiovascular complications in these two groups

The present study confirms earlier observations of hyperglycemia as an important risk factor for coronary heart disease Treatment with tolbutamide is likely to afford protection Furthermore as has been noted in the parallel studies (25 26) smoking in combination with reduced glucose tolerance appears to increase the risk of cardiovascular complications

ACKNOWLEDGEMENTS

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Plethysmographic studies in individuals with borderline glucose tolerance and varying smoking habits

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ABSTRACT

One hundred and twenty male subjects with known reduced glucose tolerance for at least 9 years were compared plethysmographically with 44 male control subjects The borderline subjects had been randomized to 4 different groups. The first group was given no treatment at all the second one was given dietary instructions the third dietary instructions and placebo tablets and the fourth dietary instructions and tolbutamide, 500 mg 3 times daily

Mean values for flow pulsations and first flow during reactive hyperemia were lower in the untreated borderline group than in the controls. Dicrotic flow waves were significantly lower in the borderline groups than in the controls

The untreated borderline group had lower flow pulsations first flow and dicrotic flow values than the other borderline groups. The differences were however rather small and seldom significant.

In the non-smokers the plethysmographic differences between all groups were rather small apart from dicrotic flow waves, in which significant differences were found between borderline subjects and controls.

The smokers of all groups had considerably lower plethysmographic values than the corresponding non-smokers the differences being more pronounced in the heavy smokers Unmeasurable dicrotic flow waves were significantly more frequent in the smokers (17 of 91) than in the non-smokers (2 of 73) Among the smokers the lowest plethysmographic values were seen in the untreated borderline group The difference in first flow between the untreated group and the tolbutamide group was significant as well as the difference in dicrotic flow between the untreated and the control group

Thus, the only plethysmographic measurement which separated borderline subjects from controls independent of smoking habits appeared to be the dicrotic flow wave.

The lowest plethysmographic values were found in the untreated group while the plethysmographic values of the tolbutamide group were close to those of the controls.

INTRODUCTION

In the Tecumseh study (19) patients with coronary cerebral or peripheral vascular disease electrocardiographic T wave changes or hypertension had higher blood glucose levels than persons of the same sex and age in the total population. Similarly it has been found (11 12 13) that patients with atherosclerotic vascular disease in the leg arteries had abnormal glucose tolerance related to the severity of the arterial disease. In prospective studies (10 20) subjects with borderline glucose tolerance developed more "arterial" symptoms and electrocardiographic abnormalities than control subjects, but less than patients with diabetic glucose tolerance curves.

individuals were asked not to smoke on the day of examination. Blood samples were collected for analysis of sedimentation rate haemoglobin number of red cells packed red cell volume glucose aspartate-aminotrans- ferase alanine-aminotransferase cholesterol, triglycerides potassium, uric acid and creatinine. A urine sample was tested for the presence of glucose or albumin

Each individual was carefully examined by the same physician with special reference to the circulatory system. Heart rate and blood pressure were measured in supine position after 15 minutes of rest. The individuals were asked for cardiovascular symptoms using a standardized questionnaire (28)

The plethysmographic study was made out 2 hours after lunch in a room with constant temperature through the year (22–23°C) An air filled plethysmograph with a pneumotachograph as the measuring device was applied on both calves (17) All recordings were done with the individual lying on the back with slightly flexed knees and with the centre of the calf segment about 7 cm above heart level. Occlusion cuffs were placed around the lower third of the thighs.

Two types of recordings were made 1) pulsatile changes of flow in the segment and 2) arterial inflow following sudden venous occlusion of 50 mm Hg on the thigh. For the first type of recording a pressure of 30 mm Hg was constantly applied to the thigh to prevent retrograde venous pulsations (6 17) The primary signal from the pneumotachograph – the rate of volume change – then represents arterial flow in and out of the segment venous outflow being constant. The integrated flow curve represents changes in segment volume. The maximal difference in volume corresponding to systole and diastole was measured. However these volume pulsations did not provide more information than the measurements on the primary flow signal. The flow pulsations were defined as the difference between the highest inflow and the highest outflow rates i.e. the maximal amplitude of the flow curve (Fig 1) (6 17).

Feinberg and Lax (4) described a qualitative abnormality of the pulse wave in diabetes, consisting of a progressive diminution or disappearance of the dicrotic wave These changes could be analysed quantitatively with the present technique (Fig 1) The dicrotic flow was defined as the amplitude of the upward deflection of the flow curve in early diastole This represents actual inflow and/or reduced outflow rate In some individuals there was no discernible dicrotic inflow wave these individuals were said to have a dicrotic flow ≤0

The resting flow and the first and maximal flow after 3 minutes of arterial occlusion (to 250 mm Hg) were recorded with a venous stasis of 50 mm Hg on the thigh as in conventional venous occlusion plethysmography (6 17) The resting flow did not differ between the groups and was not analysed further

In all individuals both legs were examined but only the values from the right leg were used for calculations. In a few cases the values from the left leg were chosen because of technical deficiencies on the right side. The maximal flow gave the same information as the first flow and the values are therefore not given in the tables

Conventional statistical methods were used. The significance of differences between groups was evaluated with Student's t-test and Chi²-test. The Wilcoxon rank sum test was also used for assessing the significance of differences in the dicrotic flow

RESULTS

Some characteristics of the different groups in 1971–1973 are described in the preceding paper (22 Table II) The mean age of the groups varied between 50 and 59 years. The treated borderline groups were older than

It is thus possible that subclinical abnormalities in glucose tolerance may precede atherosclerosis. This raises the important issue whether it would be possible to prevent atherosclerosis in such individuals by dietary regulations or treatment with antidiabetic drugs. The general opinion is that accurate treatment of the carbohydrate metabolism in diabetes mellitus offers some protection against diabetic angiopathy and atherosclerosis (2, 7, 21). By contrast, the U.G.D.P. study (28) suggested that treatment with tolbutamide accelerates the development of cardiovascular complications. However, Keen et al. (8, 9) found that patients with "borderline diabetes" treated with tolbutamide developed fewer vascular events than control groups.

The purpose of the present study is to examine the occurrence of plethysmographic abnormalities in individuals with reduced glucose tolerance. The studies by e.g. Feinberg et al. (4, 14, 15) and Woolam et al. (29) suggest that plethysmographic observations may be a sensitive index for detecting vascular complications in diabetes.

A group of subjects with borderline glucose tolerance has been followed over nearly 10 years (22). Individuals without treatment and with different types of treatment, including tolbutamide, were examined. A control group with normal glucose tolerance was also studied.

The plethysmographic findings were also related to other factors, e.g. blood lipid values. As smoking influences the peripheral circulation, the material was also subdivided according to smoking habits (5, 23).

MATERIAL

In a diabetes detection survey in Malmöhus county in 1962–1965, 228,853 individuals (82% of the population) were screened for glucosuria with Clinistix® after a carbohydrate rich meal (1).

Glucosuria was found in 2477 individuals. Of these 2180 were further examined with an oral glucose tolerance test (30 g glucose per m² body surface) during standardized conditions. Using criteria given by Scherstén et al. (16), 578 individuals with borderline glucose tolerance were found. Some of these were randomly selected and divided into 4 equal groups. The first group was not treated at all, the second one was given dietary instructions, the third dietary instructions and placebo tablets and the fourth group dietary instructions and tolbutamide, 0.5 g 3 times daily (22, 23). These 4 groups are referred to as "untreated", "diet", "placebo" and "tolbutamide" groups respectively. At the same time age matched controls with no family history of diabetes and with normal glucose tolerance, were selected at random from the entire screened population. Most of the borderline subjects and the controls have been followed during 10 years.

The classification of the individuals in the present study is thus based on the glucose tolerance values in 1962–1965. The present study was restricted to men aged 50 to 67 at the follow up in 1972–1973.

The material was also grouped according to smoking habits. With a few exceptions the subjects had unchanged smoking habits during the observation period. A few individuals that stopped smoking were excluded from the study.

Of the original 177 subjects with borderline glucose tolerance and of the 46 control subjects, 122 borderline subjects and 44 controls remained for the plethysmographic study in 1972–1973. The reasons why some subjects were excluded are given in Table I of the preceding paper (22).

METHODS

The examinations started at 8 o'clock in the morning. Eating or drinking were not allowed from 10 o'clock p.m. the day before. The

individuals were asked not to smoke on the day of examination. Blood samples were collected for analysis of sedimentation rate, hemoglobin, number of red cells, packed red cell volume, glucose aspartate-aminotransferase, alanine-aminotransferase cholesterol, triglycerides, potassium uric acid and creatinine. A urine sample was tested for the presence of glucose or albumin.

Each individual was carefully examined by the same physician with special reference to the circulatory system. Heart rate and blood pressure were measured in supine position after 15 minutes of rest. The individuals were asked for cardiovascular symptoms using a standardized questionnaire (26).

The plethysmographic study was made about 2 hours after lunch in a room with constant temperature through the year (22–23°C). An air filled plethysmograph with a pneumotachograph as the measuring device was applied on both calves (17). All recordings were done with the individual lying on the back with slightly flexed knees and with the centre of the calf segment about 7 cm above heart level. Occlusion cuffs were placed around the lower third of the thighs.

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Conventional statistical methods were used. The significance of differences between groups was evaluated with Student's t-test and Chi²-test. The Wilcoxon rank sum test was also used for assessing the significance of differences in the dicrotic flow.

RESULTS

Some characteristics of the different groups in 1971–1973 are described in the preceding paper (22 Table II). The mean age of the groups varied between 50 and 59 years. The treated borderline groups were older than

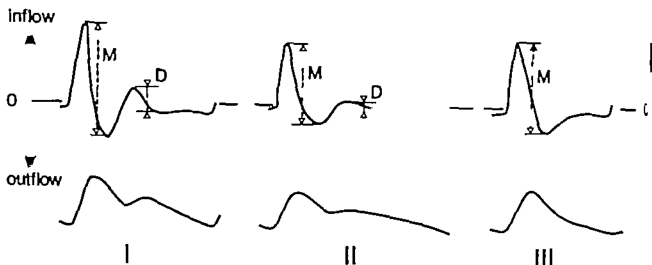


Fig. 1. Three representative pulsation curves

Upper row: Flow pulsations. Arrows indicate the way of measuring maximal amplitude and dicrotic flow wave (M and D respectively).

Lower row: Volume pulse ... the integrated flow signal. Subject I shows a large dicrotic flow wave, subject II a small one and subject III no measurable dicrotic wave.

TABLE I *Heart rate and blood pressure*

Mean values ± S E M

Group	No of subjects	Heart rate (beats/min)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Pulse pressure (mm Hg)
Borderline groups					
Untreated	41	74 4 ± 1 69	145 3 51	90 ± 1 5	56 2 6
Diet	32	70 9 ± 1 96	145 2 78	88 1 5	58 ± 2 1
Placebo	30	64 9 ± 2 32	146 4 26	88 1 8	59 3 3
Tolbutamide	27	69 9 ± 1 93	139 ± 2 34	85 ± 1 2	54 2 8
Controls	44	67 1 ± 1 74	135 1 86	86 ± 1 1	50 1 3

TABLE II *Plethysmographic observations*

Mean values ± S E M

Group	No of subject	Flow pulsations (ml min^{-1} 100 ml^{-1})	First flow (ml min^{-1} 100 ml^{-1})	Dicrotic flow (% of flow pulsations)	Dicrotic flow % (no of subjects)
Borderline groups					
Untreated	41	44 6 1 67	22 2 0 94	5 1 ± 0 38	5
Diet	32	45 4 2 06	22 9 1 00	5 9 ± 0 45	6
Placebo	30	50 9 ± 2 33	23 8 1 31	5 9 0 41	2
Tolbutamide	27	48 1 1 88	24 9 ± 1 27	5 1 38	1
Control	44	49 1 ± 1 70	25 9 1 07	8 3 0 63	5

the control group ($p<0.05$) There were no important differences in height or weight. Fasting blood glucose values did not differ between the groups. The cholesterol values in the untreated and diet groups were higher than in the controls ($p<0.05$) Triglycerides in the tolbutamide group were lower than in the other groups ($p<0.05$) except for the placebo group.

There were no significant differences between smokers and non-smokers as regards the variables given in Table II of the preceding paper (22)

The results of the glucose tolerance tests and of the other laboratory data such as hemoglobin, uric acid potassium aspartate- and alanine-aminotransferase and creatinine will be presented in detail elsewhere (25)

Table I shows mean values for heart rate and blood pressure in the different groups. The untreated group had higher heart rate than the controls and the placebo group ($p<0.01$) Systolic blood pressure in all borderline groups was higher than in the control group However the difference was not significant for the tolbutamide group The diastolic pressure in the untreated group was higher than in the tolbutamide group and the controls ($p<0.05$). In all borderline groups pulse pressure – like the systolic pressure – was higher than in the controls ($p<0.01$).

There were no significant differences between smokers and non-smokers as regards heart rate and blood pressure.

Table II shows the plethysmographic observations in the entire material, regardless of smoking habits

It will be seen that the untreated borderline group had lower values than the other groups There is a tendency for flow pulsations and first flow to be higher with more "active treatment. In the tolbutamide group the values are close to those of the controls. However only a few differences between untreated patients and the other groups were significant. Thus, the untreated group had significantly lower flow pulsations than the placebo group ($p<0.05$) The controls had higher first flow than the untreated group ($p<0.05$).

The dicrotic flow expressed in absolute terms, or as per cent of the flow pulsations (Table II) was clearly higher in the controls than in all borderline groups. The values in the different treatment groups were about equal and significantly different from those in the control group ($p<0.05$–0.01).

In the non-smokers (Table III) there were no significant differences between any of the groups as regards flow pulsations and first flow In particular it should be noted that the untreated borderline group had values of the same magnitude as both the tolbutamide and the control group. Again, the dicrotic flow values are outstanding the mean value for the control group being significantly higher than those of all the other groups ($p<0.05$–0.01). Between the borderline groups there were no significant differences.

In the smokers (Table IV) there was again the same tendency for the untreated borderline group to have lower values than the other groups. Significant differences were observed between the tolbutamide and the control groups in flow pulsations ($p<0.05$) and between the tolbutamide and the untreated groups in first flow ($p<0.05$). The controls had higher values for dicrotic flow but the only significant difference was that between the controls and the untreated borderline group ($p<0.05$). Among the various borderline groups there were no significant differences. Several of the smokers had dicrotic flow values, which were not measurable by the present technique (i.e. dicrotic flow values were equal to, or less than 0). Thus among the 91 smokers there were 17 with unmeasurable dicrotic flow whereas in the 73 non-smokers there were only ? with unmeasurable dicrotic flow the difference being significant.

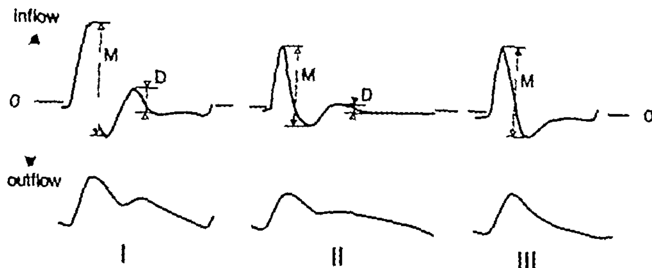


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Upper row: Flow pulsations. Arrows indicate the way f measuring ma imal amplitude and dicrotic flow wave (M and D respectively)

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TABLE I Heart rate and blood pressure

Mean values ± S E M

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Tolbutamide	27	69 9 ± 1 93	139 ± 2 36	85 1 2	54 ± 2 0
Controls	44	67 1 ± 1 74	135 ± 1 86	86 ± 1 1	50 ± 1 1

TABLE II Plethysmographic observations

Mean values ± S E M

Group	No f sub-ject	Flow pulsations (ml min.$^{-1}$ 100 ml^{-1})	First flow (ml min.$^{-1}$ 100 ml^{-1})	Di ro i flow (f flow pulse ione)	Dicrot flow M 0 (no f subject)
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Placebo	20	50 9 2 31	23 8 ± 1 51	5 9 ± 0 1	2
Tolbutamide	27	48 1 1 88	24 9 ± 1 27	5 1 ± 0 38	1
Controls	44	49 1 ± 1 70	25 9 ± 1 07	6 1 0 63	5

The effect of smoking on the plethysmographic variables is further demonstrated in Table V which shows the material grouped in non-smokers, light smokers (<15 grams per day) and heavy smokers (≥15 grams per day). The treated borderline groups are combined, since otherwise the number of subjects in each subgroup would have been too small for analysis. It may be seen that in untreated and treated borderline groups, as well as in the controls, there was a clear relation between smoking habits and reduction in flow pulsations, with significantly lower values in heavy smokers than in non-smokers ($p<0.05$).

Similarly the first flow values were lower in the smokers. The differences between non-smokers and heavy smokers were significant in all groups ($p<0.05$) except in the heavy smoking controls. From Table V it also appears that the relation between smoking and dicrotic flow as measured here is not so clear-cut as its effects on the other variables mentioned. Again, it should be pointed out that unmeasurable dicrotic flow values were more frequent among the smokers.

Attempts were made to find significant correlations between plethysmographic measurements on the one hand and other variables such as age, body weight, smoking, cholesterol, triglycerides, blood glucose, heart rate, blood pressure, on the other hand. The relationship between different variables has been examined by linear correlation but besides multivariate analysis was used and partial correlation coefficients between the different variables mentioned above were calculated. In all groups there was a significant correlation between blood pressure measures (systolic, diastolic blood pressure and pulse pressure) ($p<0.001$) and also between first flow and maximal flow ($p<0.001$). Otherwise only a few significant correlations were found and the tendencies varied in the different groups.

Using linear correlation there was found a decrease in flow pulsations, first flow ($p<0.05$) and dicrotic flow with rising age but only in the untreated borderline group. The greatest decrease was found in the smokers. Decreases in flow pulsations, first flow and dicrotic flow were significantly correlated to blood glucose values in the control group.

In the preceding paper are given the results of exercise tests in the same patient groups as studied plethysmographically here (22). There was no apparent correlation between plethysmographic variables and the ECG changes 4 minutes after exercise.

DISCUSSION

The primary aim of the present study is to analyse plethysmographic findings in subjects with borderline glucose tolerance. The material was selected at random from a diabetes detection survey in 1962–1965 and has been followed for a long time. During the follow-up period the material was reduced in various ways. Thus 12 of the original 177 patients died (8 in coronary heart disease and 4 of other causes), 25 developed diabetes and 4 intermittent claudication. This naturally means some selection, since plethysmographic observations in these 41 patients may have been different from those in the remaining patients.

Development of diabetes, cardiovascular complications and mortality will be analysed in a separate study involving a larger series of patients (24).

The plethysmographic variables studied here can be influenced by several factors. The pulsatile flow changes, particularly flow pulsations, are influenced by the pressure in the main artery of the leg as well as by the visco-elastic properties of the local arterial tree. Central circulatory factors, e.g. cardiac function, aortic elasticity and total peripheral vascular resistance may also affect the flow

TABLE III *Plethysmographic observations in non-smokers*

Mean values ± S E M

Group	No of sub-jects	Flow pulsations (ml min.$^{-1}$ 100 ml^{-1})	First flow (ml min.$^{-1}$ 100 ml^{-1})	Dicrotic flow (% of flow pulsations)	Dicrotic flow ≤ 0 (no of subjects)
Borderline groups					
Untreated	17	50 8 ± 2 73	27 1 1 90	5 7 ± 0 66	1
Diet	10	50 1 2 41	22 9 ± 1 67	6 1 1 10	0
Placebo	11	52 2 2 48	24 1 1 21	6 7 ± 0 44	1
Tolbutamide	13	48 0 ± 2 91	24 7 ± 1 92	5 4 ± 0 58	0
Control	22	52 0 ± 2 34	28 1 1 68	9 1 0 89	0

TABLE IV *Plethysmographic observations in smokers*

Mean values ± S E M

Group	No of sub-jects	Flow pulsations (ml min.$^{-1}$ 100 ml^{-1})	First flow (ml min.$^{-1}$ 100 ml^{-1})	Dicrotic flow (% of flow pulsations)	Dicrotic flow ≤ 0 (no of subjects)
Borderline groups					
Untreated	24	40 6 2 23	19 9 ± 1 17	4 7 0 42	4
Diet	22	43 3 2 78	22 9 1 36	5 8 0 58	0
Placebo	9	49 2 4 32	23 5 ± 2 13	4 9 ± 0 58	1
Tolbutamide	14	48 2 ± 2 53	25 8 1 75	4 9 0 50	1
Controls	22	43 7 ± 2 75	23 0 1 35	6 9 0 82	5

TABLE V *The effect of smoking on plethysmographic variables*

Mean value ± S E M

Light smokers < 15 g/day Heavy smokers ≥ 15 g/day

Group	Smoking habits	No of sub-jects	Flow pulsations (ml min.$^{-1}$ 100 ml^{-1})	First flow (ml min.$^{-1}$ 100 ml^{-1})	Dicrotic flow (% of flow pulsations)	Dicrotic flow ≤ 0 (no of subjects)
Borderline	Non-smokers	17	50 8 2 73	25 7 1 49	5 7 0 66	1
Untreated	Light smokers	13	43 1 2 4	21 5 32	4 4 0 54	2
	Heavy smokers	11	37 8 3 76	19 1 6	5 1 0	2
Treated	Non-smok	34	51 9 1 38	25 5 1 17	6 0 4	1
	Light smokers	30	46 8 2 03	2 7 1 19	5 7 0 39	5
	Heavy smokers	15	44 4 3 48	21 1 1 29	4 7 0 6	
Controls	Non-smokers	22	52 0 2 34	28 5 1 8	9 1 0 84	0
	Light smokers	12	44 6 3 30	22 1 1 68	6 4 0 8	3
	Heavy smokers	10	40 7 4 14	24 0 1 7	7 4 1 50	2

The effect of smoking on the plethysmographic variables is further demonstrated in Table V which shows the material grouped in non-smokers light smokers (<15 grams per day) and heavy smokers (≥15 grams per day) The treated borderline groups are combined, since otherwise the number of subjects in each subgroup would have been too small for analysis. It may be seen that in untreated and treated borderline groups as well as in the controls, there was a clear relation between smoking habits and reduction in flow pulsations with significantly lower values in heavy smokers than in non-smokers ($p < 0.05$)

Similarly the first flow values were lower in the smokers The differences between non-smokers and heavy smokers were significant in all groups ($p < 0.05$) except in the heavy smoking controls. From Table V it also appears that the relation between smoking and dicrotic flow as measured here is not so clear-cut as its effects on the other variables mentioned Again, it should be pointed out that unmeasurable dicrotic flow values were more frequent among the smokers.

Attempts were made to find significant correlations between plethysmographic measurements on the one hand and other variables such as age, body weight smoking cholesterol, triglycerides, blood glucose heart rate blood pressure, on the other hand The relationship between different variables has been examined by linear correlation but besides multivariate analysis was used and partial correlation coefficients between the different ariables mentioned above were calculated In all groups there was a significant correlation between blood pressure measures (systolic, diastolic blood pressure and pulse pressure) ($p < 0.001$) and also between first flow and maximal flow ($p < 0.001$) Otherwise only a few significant correlations were found and the tendencies varied in the different groups.

Using linear correlation there was found a decrease in flow pulsations first flow ($p < 0.05$) and dicrotic flow with rising age, but only in the untreated borderline group The greatest decrease was found in the smokers Decreases in flow pulsations, first flow and dicrotic flow were significantly correlated to blood glucose values in the control group.

In the preceding paper are given the results of exercise tests in the same patient groups as studied plethysmographically here (22) There was no apparent correlation between plethysmographic variables and the ECG changes 4 minutes after exercise

DISCUSSION

The primary aim of the present study is to analyse plethysmographic findings in subjects with borderline glucose tolerance. The material was selected at random from a diabetes detection survey in 1962–1965 and has been followed for a long time. During the follow-up period the material was reduced in various ways. Thus, 12 of the original 177 patients died (8 in coronary heart disease and 4 of other causes) 25 developed diabetes and 4 intermittent claudication. This naturally means some selection since plethysmographic observations in these 41 patients may have been different from those in the remaining patients.

Development of diabetes cardiovascular complications and mortality will be analysed in a separate study involving a larger series of patients (24)

The plethysmographic variables studied here can be influenced by several factors. The pulsatile flow changes particularly flow pulsations are influenced by the pressure in the main artery of the leg as well as by the visco-elastic properties of the local arterial tree Central circulatory factors, e.g cardiac function, aortic elasticity and total peripheral vascular resistance may also affect the flow

pulsations by changing the level and variations of pressure in the main artery of the leg This pressure may also be changed by the presence of obstruction in the artery e g atherosclerosis The mode of origin of the dicrotic flow wave is also quite complex Besides reflecting the recoil of the arterial blood column against the closed aortic valves in diastole it is also likely to be affected by the factors that can influence flow pulsations notably changes in arterial wall elasticity and peripheral resistance

The first flow and the maximal flow during the hyperemia after arterial occlusion are mainly dependent upon the perfusion pressure and the minimal total resistance in the extremity segment The flow values are not influenced by arterial elasticity but may be reduced both by atherosclerosis in the main arteries and by obstructive changes in the small peripheral vessels

The present study shows disturbed arterial function in borderline subjects Changes were seen in all plethysmographic variables studied and appear to be partially counteracted by treatment The most consistent changes occurred in the dicrotic wave which was reduced in all borderline groups regardless of treatment Changes in first flow appeared less conspicuous than changes in dicrotic flow and flow pulsations

Age varied somewhat in the different groups but seemed to affect the plethysmographic results rather little within the range studied here Similarly there were differences in cholesterol and triglyceride concentrations in the different groups but no connection between serum lipids and plethysmographic values could be revealed

As mentioned above the pathophysiological background to the changes in the plethysmographic variables studied here is quite complex. Consequently the observations made do not permit conclusions about the type of mechanism that might have caused the observed changes in the borderline subjects Strandness et al (27) have pointed out that the vascular symptoms in diabetes are mainly due to atherosclerosis. Similarly the plethysmographic changes recorded here may well be due to incipient atherosclerosis in the leg arteries Nilsén (17) found that legs with arterial wall changes on angiography but without stenosis of the artery – had a mean value for flow pulsations of 31 0 ml min $^{-1}$ 100 ml^{-1} whereas in controls the corresponding value was 46 7 ml.min. $^{-1}$ 100 ml^{-1} The reduced flow pulsations in some groups in the present study can thus be caused by slight atherosclerosis in the proximal arteries However as mentioned a contribution from changes in arterial wall elasticity in the segment studied is also quite possible. The most consistent change was actually in the dicrotic flow wave which seems more liable to be affected by elastic changes. A hint at the presence of changes in elastic properties of the arterial tree is also given by the observations on pulse amplitude. This was higher in the borderline subjects than in the controls A higher stroke volume is an unlikely explanation for the higher pulse amplitude The increased pulse amplitude *per se* would tend to increase flow pulsations in an extremity segment. It is thus possible that reduction in flow pulsations is to some extent balanced by changes which lead to a higher pulse amplitude

The effect of tolbutamide treatment on the incidence of coronary and arterial disease in patients with various forms of diabetes has been much debated during the last years. Whereas some studies suggest that tolbutamide may accelerate arterial complications (28) others have demonstrated a protective effect of treatment with tolbutamide (8 9). The present study suggests that treatment with tolbutamide has at least no deleterious effect on arterial function On the contrary there is evidence that subjects treated with tolbutamide have less reduced arterial function than untreated patients. However

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A striking difference in plethysmographic findings was observed between non-smoking and smoking subjects. The non-smokers had consistently better plethysmographic values than light or heavy smokers. Furthermore, there appeared to be a form of interaction between smoking and glucose tolerance as regards flow pulsations and first flow values since the harmful effects of smoking tended to be more pronounced in untreated border line subjects than in treated subjects. The dicrotic flow values were also clearly lower in the smokers and the frequency of unmeasurable dicrotic flow values was higher in the smokers.

The effect of smoking thus tends to obscure the association between impaired glucose tolerance and plethysmographic findings. However in non-smoking border line subjects, the only significant difference

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The effect of smoking thus tends to obscure the association between impaired glucose tolerance and plethysmographic findings. However in non-smoking border line subjects the only significant difference from the controls was found in the dicrotic flow values. It is thus likely than this parameter provides a sensitive index for assessing the influence of reduced glucose tolerance on the peripheral circulation.

The role of smoking for the development of atherosclerosis has been much discussed recently. Isacsson (5) found that first calf blood flow values during reactive hyperemia were lower in smoking men. Similarly Nilsén (17) found that among control subjects studied smokers had lower values for flow pulsations. The present study confirms these observations. Furthermore it suggests that a more detailed quantitative study of the flow pulsation pattern with special emphasis on the pattern around the dicrotic incisura, will provide a sensitive way of registering the influence of smoking. Further studies should also explore the possibility that there is an interaction between smoking and impaired glucose tolerance.

ACKNOWLEDGEMENTS

The study was supported by grants from the Swedish Medical Research Council (grant 2872) the Medical Faculty University of Lund the Swedish Diabetes Association and Albert Påhlsson's Fund. Boehringer Mannheim kindly supplied tolbutamide (Artosin®) and placebo tablets

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Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica.*

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A clinical and epidemiological study with a one-year follow-up

By Vilho Konu

To my wife and children

Oy Lounaisrannikko — Turku 1977

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INTRODUCTION

Myocardial infarction in Finland represents a serious disease entity Numerous studies have shown that the incidence and m rtality of myocardial infarction are highest in the world in Finland Consequently arious internation ally recognised studies on myocardial infarc tion in the younger age group have been per formed in Finland for example the East—West Study and the North Karelian Project. Thus efforts have been made to discover the factors which lead to uch a high incidence of th disease and also to prevent the disease.

Myocardial infarction in the elderly has received considerably less attention When the Myocardial I farction Community Register in Turk came into operation on 972 as the first communally established Ischaemic Heart Disease Register in Finland, a favourable opportunity appeared to perform a large scale tudy on myocardial infarction in the elderly In the course of th work of the register it was dicovered that over 60 / of all cases of acute myocardial infarction in Turku occur in the age group of 65 years or over

Th observation has been made that myocar dial infarction in the elderly presents with different clinical features than in the younger age groups. Many old people do not experience any chest pain and the symptoms in general are more vague transient loss of consciousness vertigo vomiting and dyspnoea. The clinical signs often seem to be milder Patients with defective memory and dementia are able to move about shortly after the infarction and appear to recover and manage well. Some patients however di suddenly

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The aim of th tudy was to record the incidence of myocardial farction in the population ged 65 years or over of the Turku city area the mortality urvival r te and prognosis ver period f one year and also to cla rify the following clinical features

— the symptoms and signs and associated mortality

— the cl nical course and complications and associated mortal ty

Attention was also paid to the significance of arious socioeconomic, familiar and other risk f ctors and factors preceding the onset of infarction and to the significance of personal habits e.g. consumption of coffee sugar and fat and to the significance of the sauna.

Because numerous factors in myocardial infarction in the elderly differ from findings in the younger age group a control group consist ing of age and sex matched inhabitants of Turku was also studied.

In order to study the difference in symptomatology between elderly and younger patients a comparison between the study group and an add tional control group consisting of patients under 65 years of age was made.

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II
REVIEW OF THE LITERATURE

1 MYOCARDIAL INFARCTION

The first description of angina pectoris in recent times was given by Heberden in 1772. Herrick (1912 1919) was the first to describe the symptoms of coronary thrombosis.

The expert committee of the world health organization (WHO 1959) considers the terms coronary heart disease" nd Ischaemic her t disease" to be synonymous. The terms refer to acute or chronic heart disease which is caused by decrease or complete interruption in the blood supply to the myocardium associated with a disease process in the coronary arteries.

The same expert committee recommended the following classification of coronary artery disease n 962 1 chest pai on exertion 2 myocardial infarction recent or ld 3 intermediate forms of these 4 painless coronary artery disease

Accord ng to the committee the significance of the electrocardiogram in coronary rtery disease is clear only in the case of myocardial infarction.

Many clinicians have studied myocardial infarction without defining it. WHO has defined the criteria by which infarction may be divided into definite possible or not acute (Working G oup of Ischaemic Heart Disease Registers EURO 8201(5) 1971)

2 PREVIOUS STUDIES ON THE PREVALENCE INCIDENCE AND MORTALITY OF MYOCARDIAL INFARCTION POPULATION AND HOSPITAL STUDIES

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Peterson has made a large scale study of hospitalised patients in Washington (Peterson et al. 1972) The material consisted of 6987 patients with infarction, drawn form 25 hospitals. He observed that ge was the only factor which significantly influenced the hospital mortality The average mortality in patients less than 60 years old was 12 / and was noticeably higher in patients over 60, to the extent that it exceeded 50 / in patients over 80.

In Los Angeles a study revealed that the prevalence of coronary artery disease in men aged 40—54 was 1 5 /o and in the 55—70 year age group 6,8 / (Chapman et al. 1957)

Russek (960, 1962) examined the prevalence of ischaemic heart disease in American doctors dentists and lawyers Clinical ishaemic heart disease was present in 2,8 % of male doctors in the 40—49 year ge group and 18,4 / in the 60—69 year age group. The disease was 3 times more common in general practitioners than in dermatologists. Ischaemic heart disease was clearly more common in the professional groups which are associated w th an obviously increased stress. Numerous studies have been performed in the U.S.A. in which the incidence and prevalence of myocardial infarction hav been examined but most of these have been performed in younger patients.

In the Framingham study which began on 1 950, patients ged from 30—59 years were followed for 14 years. The material therefore included patients over 65 years old (Dawber 1961) Kannel observed that during the 14 year period, 172 men and 42 women out of a total of 5127 patients developed a myocardial infarction for the first time (Kannel 1970) Of these patients 151 men and 37 women had definite ECG evidence of myocardial infarction Of these however 22 / of the men and 30 of the women were unaware of the fact that they had had an infarction, as also were their attending doctors. These therefore, were silent infarctions. There was no age difference in the prevalence of silent infarctions.

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In th Framingham study which began on 1 1 950 patients aged from 30—59 years were followed for 14 years The material therefore included patients over 65 years old (Dawber 961) Kannel observed that during the 14 year period 172 men and 42 women out of a total of 5127 patients developed a myocardial infarction for the first time (Kannel 1970) Of these patients 15 men and 3 women had definite ECG evidence of myocardial infarction. Of these however 22 / f the men and 30 % of the women were unaware of the fact that they had had an infarction, as also were their attending doctors. These, therefore, were silent infarctions. There was no age difference in the prevalence of silent infarctions.

II

REVIEW OF THE LITERATURE

1 MYOCARDIAL INFARCTION

The first description of angina pectoris in recent times was given by Heberden in 772. Herrick (912 919) was the first to describe the symptoms of coronary thrombosis.

The expert committee of the world health organization (WHO 959) considers the terms coronary heart disease" and ischaemic heart disease" to be synonymous The terms refer to acute or chronic heart disease which is caused by decrease or complete interruption in the blood supply to the myocardium associated with disease process in the coronary arteries.

Th same expert committee ecommended the following classification of coronary artery disease in 962 1 chest pain on exertion 2 myocardial infarction recent or old 3 intermediate forms of these 4 painless coronary artery disease

According to th committee the significance of the electrocardiogram in coronary artery disease is clear only th case of myocardial infarction

Many clinicians have tudied myocardial infarction without defining it. WHO has defined the criteria by which infarction may be d vided into defin te possibl or not acute (Working Group of Ischaemic Heart Disease Registers EURO 820 (5) 1971)

2 PREVIOUS STUDIES ON THE PREVALENCE INCIDENCE AND MORTALITY OF MYOCARDIAL INFARCTION POPULATION AND HOSPITAL STUDIES

USA

Peterson has made a large scale study of hospitalised patients in Washington (Peterson et al. 972) The material consisted of 6987 patients with infarction drawn form 25 hospitals. He observed that age was the only factor which significantly influenced the hospital mortality The average mortality in patients less than 60 years old was 12 and was noticeably higher in patients over 60 to the extent that it exceeded 50 / In patients over 60.

In Los Angeles a study revealed that the prevalence of coronary artery disease in men aged 40—54 was 1 5 / and in the 55—70 year age group 8,8 / (Chapman et al. 1957)

Russek (1960 1962) examined the prevalence of ischaemic heart disease in American doctors dentists and lawyers. Clinical ishaemic heart disease was present in 2,8 / of male doctors in the 40—49 year age group and 18,4 / in the 60—69 year age group. The disease was 3 times more common in general practitioners han in dermatologists. Ischaemic heart disease was clearly more common in the professional groups which are associated w th an obviously increased stress Numerous studies have been performed in th USA in which the incidence and prevalence of myocardial infarction have been examined but most of these have been performed in younger patients.

In the Framingham study which began on 1 1 1950 patients aged from 30—59 years were followed for 14 years The material therefore included patients over 65 years old (Dawber 96) Kannel observed that during the 14 year period 172 men and 42 women out of a total of 5127 patients developed a myocardial infarction for the first time (Kannel 1970) Of these patients 151 men and 37 women had definite ECG evidence of myocardial infarction. Of these however 22 / of the men and 30 / ot the women were unaware of the fact that they had had an infarction, as also were their tending doctors. These therefore, were silent infarctions. There was no age difference in the prevalence of silent infarctions.

Johansson (1972) later made study of patients with infarction in Malmö over he period 1960—68 The material con sisted of 3249 hospital patients with an average age of 67 years (2166 men, average age 65 and 1083 women average age 72 years) The inci dence remained the same in the different age groups over th 8 year period. 165 of the male patients (7,6 /) and 37 of the female patients (12 7) were diabetics. There were 7 / male and 15 1 / female hypertensive patients. 27 of the men and 3 3 / of the women had angina pectoris A combination of angina pectoris and hypertension was present in 5,6 / of the men and in 14,8 / of the women.

The hospital mortality rate was 33 1 / (30,4 / fo men and 38 5 / for women. The aver age d ration of hosp tal stay was 20 74 ± 2,0 days (9,9± 2,82 days for men and 22,23± 3 9 for women)

The average delay from th very first symptoms of the infarction until the patients came to the hospital was 30 9 hours 35 / came 3 hours after the onset of the infarction and 2 / later than 4 hours after the onset

The incidence of infarction was lowest in A gust. There were far more cases in June with temperature the same as in August, than in February when the average temperature was much lower

Patients less than 67 years old were investi gated in the Gothenburg Heart Register (Elmfelt 1974 Vedin 1974 and Wilhelmsson 974) The proportion of elderly patients (2 men and 18 women) was very small 5 men and 2 women died. Their diagnostic criteria were stricter than those proposed by the WHO The incidence of myocardial infarction, which was calculated only in the 60—64 year age group was 135 per 00 000 per year The mortality rate in the same age group was 507 per 100 000 per year and th survival rate 628 per 100 000 per year Postmortems were performed in 90 / of the cases (Vedin 1974) Elmfelt (974) observed that with thei criteria, the sensitivity of the study decreased in the older ge group in which the ymptoms were atypical.

Hagfeld (197) has investigated the myocardial infarctions that occured during Novem ber 968 in Copenhagen. The material consist ed of 338 cases, and of these there was total of 24 cases (71) over 60 years of age (41 men and 99 women) The mortality in the 60—69 year ge group was 58 for men and 57 / for women In patients over 70 years old the mortality for men and women was 55 /. Dividing each day into three 8 hour periods he noticed that most infarctions occured be tween 4 p.m. and midnight (37 °/) The hospi tal mortality rate was 8 /. Less than half were admitted to the coronary care unit, where there was a lower mortality rate (22 /) The majority of infarctions occurred at home (84 /) 7 / occurred at work and 7 °/ in the street.

FINLAND

Vartio has studied the prevalence of myocardial infarction in Oulu Pr vincial Hospital over the period 946—1958 (Vartio 1960) His material consisted of 165 patients over 65 years old (73 women and 92 men) The male to female ratio was .2 1

Preceding chest pain was not present in 38 5 / of patients over 60 years old Chest pains preceding the infarction lasted 1—4 weeks in 14 / of patients. The most infarctions occur red in February and the least infarctions in April. The difference was statistically signif cant. 7,8 / of the infarctions occurred during the winter months and 22,8 / during the spring months. Th highest mortality (29) was in winter and lowest (22 /) in sum mer

The majority of infarctions occured at night at rest (38 7 /) and after this most occured at work during the day Patients over 65 years old had primary mortal ty rate of 38 7 compared to a mortality rate of 26,3 / in the entire material Vartio concluded that age heart failure, hypertension and shock led to an increased mortality in the early stages.

Korhonen et al. (1960) have also investigated myocardial infarction in th elderly in Central Finland Central Hospital. The material consisted f 376 patients and of these 73 men (25,6 /) and 35 women (38,4 /) were in the 60—69 year age group There were 1 (7,4 /) infarctions in the 70—79 yea age group. There was no variation in incidence between differ ent months. There was no variation either in the different quarters of the year o with temperature or atmospheric pressure.

Kuusisto et al. (1960) have examined the relationship between myocardial infarction and diabetes in Helsinki. The material consisted of 1245 patients. The primary mortality from myocardial infarction in patients aged from 60—79 was 28,8 / for men and 39,4 / for women. The prognosis was worse in women. 9 of the patients had transient glycosuria

The ages of the patients ranged from 30—79 Silent infarction did not occur in patients who had earlier suffered from angina pectoris

Silent infarctions were twice as common in diabetics compared to the others The incidence of silent infarctions in hypertensive patients was 33 % the incidence in normotensive patients being 19 % Patients with previous ECG changes were also more liable to develop a silent infarcion.

There was no appreciable difference in prognosis over a one year period between silent and definite infarctions Kannel suggests that the most useful investigation to discover silent infarctions is a routine periodic ECG registration

In the Tecumsech study the prevalence of ischaemic heart disease in 60—69 year old men was found to be about 180 per 1000 patients per year and slightly less than this in the 70—79 year age group The prevalence in women in the 60—69 and 70—79 year age groups was 165 and 110 per 1000 patients per year respectively (Epstein 1965)

Protos et al. have examined the effect of the seasons on myocardial infarction in New York (Protos et al. 1971) There was no seasonal variation in men but in women there were two peaks one in April and a second in December The women also had three peaks in mortality — in November April and May The authors suggest that the seasons may in some way influence the development of thrombosis in women The study was performed over the period 1964—67 and the results were obtained in old people

GREAT BRITAIN

Kinlen (1973) has investigated the prevalence of myocardial infarction in Oxford. The population of the area covered was about 375 000. The overall incidence in 60—69 year old men was 13 per 1000 per year and in women 3 9 per year Brown et al found that the prevalence of ischaemic heart disease in 60—69 year old men was 8.4 % (Brown et al. 1957) In a later study (1962) he established that the mortality in ischaemic heart disease is relatively lower amongst farmers and farm labourers than amongst sedentary workers. He confirmed that cigarette smoking increases the mortality in ischaemic heart disease.

WEST GERMANY

Beck et al. (1975) have examined myocardi[al] infarction in the elderly in Berlin The materi[al] consisted of 840 patients with myocardial in[-]farction in the coronary care unit over the period 1970—74 and of these 71 patients (8 %) were over 80 years old They noted the close correlation between age and mortality the latter rising sharply with increase in age The mortality in patients over 80 years old was 61 % which was 7 times greater than in those less than 50 years old. They attribute the high mortality rate largely to an increased amount of pulmonary oedema heart failure and cardiogenic shock. They proposed that mortality in coronary care unit should be lower in older patients too since disorders of rhythm and conduction could be recognised and treated promptly

ISRAEL

Medalie (1973) has studied the incidence of myocardial infarction in Israel over a 5 year period and its association with different variables age and birth place. The incidence in men over 65 years was 20,4 per 1000 per year The incidence in cigarette smokers (over packet or more a day) was 64 per 1000 per year and in non-smokers 32 per 1000 per year

Librach et al. (1976) studied myocardial infarction in 132 elderly patients. Pain was absent at the onset of infarction in more than one third of the patients. Dyspnoea heralded the attack in one fifth and cerebral symptoms in one tenth. They found that the 30 day mortality in infarction was lower when it started with pain alone than when it started with pain and other symptoms.

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The prevalence of myocardial infarction has been investigated in Malmö (Björck et al 1957 1958 Björck 1960) The average age of male patients with myocardial infarction attending for treatment in the year 1935 was 59 years and in 1959 65 years, the corresponding ages for women were 61 and 70 years. The ratio of male to female patients was 1 66 1 The incidence of myocardial infarction in the total population was estimated to be 15—20 per 10 000 inhabitants per year

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rlosclerosis were already dead by the age of 65 years. The prevalence of narrowing of the coronary arteries in women did not increase to the same extent as in men. Nevertheless in women over 65 years old the incidence was almost 50 /

4 RISK FACTORS

1 *Hypertension*

seems to be the most important of the known risk factors (Atherosclerosis Study Group 1970 Stamler et al. 1972 Stamler 1973)

In Framingham Study Kannel et al. (1971) have shown that the risk of developing a myocardial infarction is related to hypertension in both men and women A rise in both systolic and diastolic blood pressure leads to an increased risk, but the former ppears to indicate more serious risk in development of myocardial infarction.

Tibblin (1967) in a population study has shown that blood pressure increases with age that systolic pressure increases more than diastolic pressure after 50 years of age and also that elderly women have higher blood pressure than elderly men

Colandrea et al. (970) found that systolic hypertension in the elderly was no risk factor in myocardial infarction, but the cardiovascular mortality however was higher in hypertensive than normotensive patients

2 *Diabetes*

The relationship between myocardial infarction and diabetes has long been known. An increased risk of myocardial nfarction has been noticed not only in diabetics but also in patients with impaired glucose tolerance (Ostrander et al 1965 Epstein 1967) In the Framingham Study female diabetics were shown to hav a greater risk of developing myocardial infarction than male diabetics (Kannel et al. 1967) Coexistent diabetes has been observed in large proportion f patients with myocardial infarction (Sievers et al. 1961 Sievers 1963 Wahlberg 1966 Simborg 1970) It has also been shown that patients who have recovered from myocardial infarction ha e abnormal response to the intravenous glucose tolerance test more often than in controls (Wahlberg 1966)

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Kretny (1976) found in Copenhagen that treated diabetics did not have an increased risk of myocardial infarction.

3 *Lipids*

A high serum cholesterol is considered to be one of the major risks in myocardial infarction (Atherosclerosis Study Group 1970, Simborg 1970, Stamler et al. 1972 Stamler 973) The average serum cholesterol in patients with myocardial infarction is higher than in the normal population (Bjorck et al. 1957 Carlson 1960 Gustafsson et al. 1972) Prospective studies have shown that a high serum cholesterol in men is closely related to myocardial infarction statistically (Keys et al. 1970, Rosenman et al. 1970 Kannel et al. 197 West lund et al 1972)

The Framingham Study also revealed that a high serum cholesterol in women less than 50 years old was associated with a greater risk of developing myocardial infarction (Kannel et al 1971)

In the Pooling Project in th United States, the observation was made that in women with cholesterol level over 250 mg/100 ml the risk of developing myocardial infarction was twice as high as in the rest of the population (Stamler et al 972)

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The East West Study performed in Finland (Karvonen et al. 1970 Keys 1970) revealed that the cholesterol levels and also the incidence of coronary artery disease were higher in Eastern Finland than in Western Finland.

Nikkila et al. (973) and Aro (973) examined the serum lipids in 10 young patients who had recovered from myocardial infarction and in 412 close relatives They noted that the average serum cholesterol and triglyceride levels in the relatives were noticeably higher than in a control group but lower than in the patients.

Fredriksson et al. (1965) proposed that there e primary hyperlipoproteinaemias Three IV) are associated with a increas- myocardial infarction

associated with the infarction 9 % of the patients had established diabetes (6,2 % of men and 16 % of women) Hospital mortality from infarction was greater in diabetics than in previously healthy patients.

In Kuusisto s series 11 5 % of men and 13 1 % of women had painless infarctions. Of these patients 8 were male diabetics and 21 female diabetics (28,8 %) There was a small peak in the prevalence of infarction in September There were no significant peaks in winter

Ruikka et al (1966) made a population study of people aged 65 years or more in Turku. The series consisted of 481 subjects selected at random and out of these 4 1 % of the men and 1 % of women were aware of myocardial infarction. However ECG changes consistent with myocardial infarction were found to be present in 11 % of the men and 10 % of the women.

Karvonen et al. (1970) have shown that Eastern Finland has the world s highest incidence of myocardial infarction. Consequently a more detailed study of myocardial infarction in North Karelia was undertaken connected with an attempt to prevent its occurrence. (Puska et al. 1973) It was noticed that the incidence of myocardial infarction was highest in men in the age group of 70—74 years and in women 80—84 years

Kalliomäki et al. (1960) studied the association between myocardial infarction and bundle branch block (BBB) The series consisted of 141 patients the majority of whom were elderly There were 59 patients in the 60—69 year age group and a further 53 patients were aged 70 or older LBBB was present in 84 patients (60 %) and RBBB in 57 (40 %) BBB was transitient in 7 cases (5 %) During hospitalisation 15 patients (8 %) with myocardial infarction and RBBB died, the mortality rate in patients with LBBB and myocardial infarction was 32 1 %. The corresponding death rate in patients with myocardial infarction without associated cardiac arrhytmias or conduction disturbances was 16,2 %.

3 POST MORTEM STUDIES

Bertolini et al (1966) have investigated a total of 274 cases of infarction in patients over 55 years old in the year 1961 1962 1963 and 1964 in Milan There were 104 cases of infarction in the 65—74 year age group and of these 46 (44 %) were undiagnosed In the over 75 year age group these were 73 cases of infarction 44 (60 3 %) of which were undiagnosed. The authors stressed that an ECG is mandatory in the investigation of elderly patients wih cardiorespiratory distress.

Uotila (1945 1970) has made an extensive study of the total number of post-mortems performed for medicolegal reasons during the years 1923—42 at the Department of Forensic Medicine of Helsinki University He observed that disease of the heart and aorta accounted for 59 6 % of all deaths and of these 62 9 % were due to myocardial infarction. The corresponding figures for the years 1966—67 were 85 and 79 % respectively revealing a relative increase in the cases of infarction.

In a post mortem investigation Möttönen (1969) has studied the incidence of myocardial infarction in Finland. The material consisted of a total of 1756 patients (1093 men and 663 women) The average age of men was 62 years and women 69,8 years. The author noticed that patients in North Karelia and Northern Finland who had suffered a myocardial infarction were younger than those in Western Finland or Helsinki. The incidence of recent septal or anterior wall infarcts was almost significantly greater in North Karelia as compared to Western Finland. Men with recent infarcts found to be more obese than those with old infarcts or coronary sclerosis In women the relative body weight was also greater in those who died from infarction The heart weight in men was greater in those who died from infarction than from other causes of death.

Rissanen (1969) examined the changes in the coronary arteries occurring in patients dying from either coronary artery disease or accidental causes He observed that arteriosclerotic changes in the coronary arteries of Finns appeared at the end of the second decade in men while in women these changes appeared 10 years later Arteriosclerotic changes were present in almost all of the coronary arteries in men over 35 years old and in women over the age of 45 Coronary artery changes causing a narrowing of one or more of the vessels by over 50 % were present in one third of the men aged 45—54 years and in half of the men aged 55 years. A remarkable feature of this study was the fact that there was no appreciable increase in the prevalence of coronary artery disease from the 55—64 year age group to the over 65 year age group. This finding may have resulted from the fact that some of these men with a marked degree of coronary arte

rosclerosis were already dead by the age of 65 years. The prevalence of narrowing of the coronary arteries in women did not increase to the same extent as in men. Nevertheless in women over 65 years old the incidence was almost 50 %.

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Ruikka et al. (1966) made a population study of people aged 65 years or more in Turku The series consisted of 481 subjects selected at random and out of these 4 1 % of the men and 1 % of women were aware of myocardial infarction. However ECG changes consistent with myocardial infarction were found to be present in 11 % of the men and 10 % of the women.

Karvonen et al. (1970) have shown that Eastern Finland has the world s highest in cidence of myocardial infarction. Consequently a more detailed study of myocardial infarction in North Karelia was undertaken connected with an attempt to prevent its occurrence. (Puska et al. 1973) It was noticed that the incidence of myocardial infarction was highest in men in the age group of 70—74 years and in women 80—84 years.

Kalliomäki et al. (1960) studied the associa tion between myocardial infarction and bundle branch block (BBB) The series consisted of 141 patients the majority of whom were elderly There were 59 patients in the 60—69 year age group and a further 53 patients were aged 70 or older LBBB was present in 84 patients (60 %) and RBBB in 57 (40 %) BBB was transitient in 7 cases (5 %) During hospitalisation 15 patients (8 %) with myocardial infarction and RBBB died, the mortality rate in patients with LBBB and myocardial infarction was 32 1 %. The corresponding death rate in patients with myocardial infarction without associated car diac arrhytmias or conduction disturbances was 16 2 %

3 POST MORTEM STUDIES

Bertolini et al (1966) have investigated a total of 274 cases of infarction in patients over 55 years old in the year 1961 1962 1963 and 1964 in Milan There were 104 cases of in farction in the 65—74 year age group and of these 46 (44 %) were undiagnosed. In the over 5 year age group these were 73 cases of in farction, 44 (60 3 %) of which were undiag nosed. The authors stressed that an ECG is mandatory in the investigation of elderly pa tients wih cardiorespiratory distress

Uotila (1945 1970) has made an extensive study of the total number of post mortems performed for medicolegal reasons during the years 1923—42 at the Department of Forensic Medicine of Helsinki University He observed that disease of the heart and aorta accounted for 59 6 % of all deaths and of these 62,9 % were due to myocardial infarction The cor responding figures for the years 1966—67 were 85 and 79 % respectively revealing a relative increase in the cases of infarction.

In a post mortem investigation Möttönen (1969) has studied the incidence of myocardial infarction in Finland. The material consisted of a total of 1756 patients (1093 men and 663 women) The average age of men was 62 years and women 69,8 years. The author noticed that patients in North Karelia and Northern Fin land who had suffered a myocardial infarction were younger than those in Western Finland or Helsinki. The incidence of recent septal or anterior wall infarcts was almost significantly greater in North Karelia as compared to West ern Finland. Men with recent infarcts found to be more obese than those with old infarcts or coronary sclerosis In women the relative body weight was also greater in those who died from infarction The heart weight in men was greater in those who died from infarction than from other causes of death.

Rissanen (1969) examined the changes in the coronary arteries occurring in patients dying from either coronary artery disease or acciden tal causes. He observed that arteriosclerotic changes in the coronary arteries of Finns appeared at the end of the second decade in men while in women these changes appeared 10 years later Arteriosclerotic changes were present in almost all of the coronary arteries in men over 35 years old and in women over the age of 45 Coronary artery changes causing a narrowing of one or more of the vessels by over 50 % were present in one third of the men aged 45—54 years and in half of the men aged 55 years A remarkable feature of this study was the fact that there was no appre ciable increase in the prevalence of coronary artery disease from the 55—64 year age group to the over 65 year age group This finding may have resulted from the fact that some of these men with a marked degree of coronary arte

rioscleroals were already dead by the age of 65 years. The prevalence of narrowing of the coronary arteries in women did not increase to the same extent as in men. Nevertheless in women over 65 years old the incidence was almost 50 /s.

4 RISK FACTORS

1 Hypertension

seems to be th most important of the known risk factors (Atherosclerosis Study Group 1970 Stamler et al. 1972 Stamler 973)

In Framingham Study Kannel et al. (1971) have shown that the risk of developing a myocardial infarction is related to hypertension in both men and women. A rise in both systolic and diastolic blood pressure leads to an increased risk, but the former appears to indicate a more serious risk in development of myocardial infarction.

Tibblin (1967) in a population study has shown that *blood pressure increases with age* that systolic pressure increases more than diastolic pressure after 5 years of age and also that elderly women have higher blood pressure than elderly men.

Colandrea et al. (970) found that systolic hypertension in the elderly was no risk factor in myocardial infarction but the cardiovascular mortality however was higher in hypertensive than normotensive patients

2 Diabetes

The relationship between myocardial infarction and diabetes has long been known. An increased risk of myocardial infarction has been noticed not only in diabetics but also in patients w th impaired glucose tolerance (Ostrander et al 1965 Epstein 967) In the Framingham Study female diabetics were shown to have a greater risk of developing myocardial infarction than male diabetics (Kannel et al. 1967) Coexistent diabetes has been observed in a large proportion of patients with myocardial infarction (Stevers et al. 96 Sievers 1963 Wahlberg 1966 Simborg 1970) It has also been shown that patients who h ve recovered from myocardial infarction have bnormal response to the intravenous glucose tolerance test more often than in controls (Wahlberg 1966)

Tibblin (1967) has observed that a rise in the fasting blood sugar does not lead to an increased risk of myocardial infarction. The author has studied the incidence and not the *prevalence* as in the Tecumseh Study (Epstein 1967)

Kretny (1976) found in Copenhagen that treated diabetics did not have an increased risk of myocardial infarction.

3 Lipids

A high serum cholesterol is considered to be one of the major risks in myocardial infarction (Atherosclerosis Study Group 1970, Simborg 1970 Stamler et al. 1972 Stamler 1973) The average serum cholesterol in patients with myocardial infarction is higher than in th normal population (Björck et al. 1957 Carlsson 1960 Gustafsson et al. 1972) Prospective studies have shown that a high serum cholesterol in men is closely related to myocardial infarction statistically (Keys et al. 1970, Rosenman et al. 1970 Kannel et al. 1971 Westlund et al. 972)

The Framingham Study also revealed that a high serum cholesterol in women less than 50 years old was associated with a greater risk of developing myocardial infarction (Kannel et al. *1971*)

In the Pooling Project in the United States, the observation was made that in women with cholesterol level over 250 mg/ 00 ml the risk of developing myocardial infarction was twice as high as in the rest of the population (Stamler et al. 1972)

Carlsson et al. (1972) demonstrated a linear relationship between the serum consentrations of both cholesterol and triglyceride and the risk of developing myocardial infarction.

Kasanen et al. (1963) have also observed that a high serum cholesterol increases the risk of myocardial infarction in patients less than 60 years old.

The East West Study performed in F nland (Karvonen et al. 1970, Keys 1970) revealed that the cholesterol levels and also the incidence of coronary artery disease were higher in Eastern Finland than in Western Finland.

Nikkilä et al. (1973) and Aro (1973) examined the serum lipids in 101 young patients who had recovered from myocardial infarction and in 412 close relatives. They noted that the average serum cholesterol and triglyceride levels in the relatives were noticeably higher than in control group but lower than in the patients.

Fredrikson et al. (1963) proposed that there are five primary hyperlipoproteinaemias. Three of these (II—IV) are associated with a increased risk of myocardial infarction

4 Physical inactivity
has also been suggested to be a risk factor in myocardial infarction (Simborg 1970 Karvonen 1972 Stamler 1973) Results have however not been quite as significant as in the case of the other risk factors The results obtained in both the Health Insurance Plan (Frank et al. 1966) and in the Framingham Study (Kannel 1966) have revealed that the incidence of fatal myocardial infarction is significantly increased in physically inactive men. The Framingham Study did not show any such association with angina pectoris. Angina pectoris was shown to be more common in young men in the Health Insurance Plan. In the Western Collaborative Study (Frank et al 1970) it was demonstrated that in association of regular body exercise fatal myocardial infarction was rarer On the other hand in the Seven Countries Study (Keys et al. 1970) of Finland which has the highest incidence on association between myocardial infarction and physical inactivity was observed. It is also worth mentioning that in North Karelia the majority of patients with myocardial infarction were neither physically inactive nor obese.

5 Smoking habit
A strong association between cigarette smoking and non fatal infarction has been found but the relationship between angina pectoris and smoking is not clear (Jenkins et al. 1968 Selzer 1969 Simborg 1970) Pipe and cigar smoking are not associated with an increased mortality from myocardial infarction (Fletcher et al. 1970) An increased mortality from myocardial infarction has also been observed in women who smoke.

The increased risk statistically associated with smoking has been found to decrease with age until there is no excessive risk of coronary heart disease in smokers over 75 years. (Selzer 1966)

The mode of action of smoking has been investigated. Fletcher et al (1970) and Rose (1973) have proposed that in the presence of a reduced coronary blood flow carbon monoxide and nicotine aggravate myocardial ischaemia and may increase the risk of arrhythmias through the effect of nicotine induced release of catecholamines. The association between atherosclerosis and smoking (Auerbach et al. 1965 Rissanen et al. 1972) suggests that the latter has also a chronic effect. The first 5 year follow up studies involving almost 13.000 men from seven countries (Keys et al. 1970) demonstrated an association between smoking and mortality from myocardial infarction in the United States This association was not, however demonstrated in the other countries, including Eastern Finland, which had the highest mortality from myocardial infarction in men

6 Obesity
Overweight is generally considered to increase somewhat the risk of myocardial infarction even though the association remains unclear In the Tecumseh study the prevalence of myocardial infarction was found to be dependent on overweight in men but not in the case of women (Epstein 1965) An association between overweight and an increased risk of sudden death in patients with angina pectoris was noticed in the Framingham Study However there was no association with myocardial infarction (Kannel et al. 1967) Multivariate computer analysis in the Seven Countries Study (Keys et al. 1972) showed that relative weight or obesity did not contribute to the development of myocardial infarction when the factors of age blood pressure serum cholesterol and smoking were comparable. The material in Bjurulhjelm s post mortem study (1959) consisted of 110 patients aged from 25—88 years 19 of whom had suffered a myocardial infarction. The author demonstrated that the degree of coronary artery sclerosis was correlated to the size of the subcutaneous fat cell

Bjorntorp et al (1971) proposed that two forms of obesity exist. One form is characterized by hypertrophy of fat cells and leads to moderate obesity This type is associated with metabolic disturbances. The other form has an increased number of fat cells and is associated with much more severe obesity but less cardiovascular complications. Other investigator however have failed to find any difference in the amount of body fat or size of fat cells when compared to control groups (Berchtold et al. 1972 Bengtsson 1973) Cramer et al (1966) did not observe a relationship between obesity and coronary artery changes as demonstrated by coronary angiography

III

MATERIAL AND METHODS

1 AREA COVERED AND POPULATION STUDIED

The City of Turku

Turku is the third largest city in Finland. It is situated on the south—west coast 60 27 1N 22 16 1E The total area of Turku in 1972 was 244.25 km² Th north—south length of the city area was 27 km and the east—west breath 16 km. Turku is an industrial and commercial centre and there are two universities The average temperature in the year 1972 was 5,8 C and in 1973 5 1 The highest recorded temper ture in 1972 was 31,0 C and the lowest temperature that occured during the study was —21 7 C in the winter of 1973 The average relative humidity in 1972 was 81 %. The population of Turku in 1972 was 158 167 and in 19 3 163 130

Inhabitants ov the age of 65 in Turku city 973

Age	65—69	70—74	75—79	80—84	85—89	90—	total
men	2669	1598	8 3	32	105	26	5532
women	4465	3209	2087	1090	43	95	11377
total	7 34	4807	2900	14	536	1	16909

The percentage of persons over 65 years old of he total population of Turku was 10,36 %. Of these 32 were men and 67 3 were women

H alth services

In 97 there were approximately 250 physicians working in Turku. Only a few of them had a whole-day private practice.

I Finland patients who have acute chest pain come traditionally straight to hospital emergency ward

Turku has two hospitals with department of internal medicine. Patients with myocardial infarction are generally taken to Turku University Central Hospital which is on duty day and night. The second hospital is Turku City Hospital, whose out-patient department is attended mainly by geriatric patients. Patients with chest pain may also present at this hospital during the day-time The department of internal med icine in Turku City Hospital has 478 beds. In-patients may develop myocardial infarction on the ward following an admission for an entirely different complaint Such patients are naturally treated in this hospital and only rarely is it necessary to transfer a patient to Turku University Central Hospital.

The third hospital is Paimio Hospital, where are mainly patients with tuberculosis and other lung diseases.

Coronary care unit (CCU) In CCU in Turku University Central Hospital are 7 beds for men and 3 beds for women. There is no cardiac ambulance (i.e. containing resuscitation equipment and staffed by doctors) However the ambulance service is quick because the distances involved are short, and the patients can therefore be admitted for treatment n a relatively short time at Turku University Central Hospital.

2 HEART REGISTER

Myocardial Infarction Community Register in Turku (Turku Heart Register) was established at the beginning of 1972 according to a programme prepared at the regional office of WHO (Working Group of Ischaemic Heart Disease Registers EURO (1) 1968 (2) 1969 (4) 1970, and (5) 1971

The purpose of the register is to compile more detailed information on acute ischaemic heart disease, myocardial infarction and

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	80—84	85—89	90—	total
	32	05	6	553
	090	431	95	377
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The purpose of the register is to compile more detailed information on acute ischaemic heart disease myocardial infarction and

sudden death. In accordance with the directions of the WHO any patient with suspected myocardial infarction or sudden coronary death should be recorded in the register

A clinical and epidemiological study dealing with coronary disease in the elderly began on 1 3 1972 and lasted 14 months until 30.4 1973

The personel consisted of one nurse and three part time physicians The registry had its own office in the Tuberculosisbureau, where roentgenograms and record forms were filled and the follow up study was performed

All the hospitals and physicians in Turku were notified of the commencement of operation of the heart register The physicians were requested to report patients with myocardial infarction to the registry Circulars were sent and personal visits made to the relevant departments of both Turku University Hospital and Turku City Hospital. Paimio Hospital was also notified. The cases of sudden coronary death were recorded from the death certificates collected by the city health officer The Turku Heart Registry therefore checked all the death cetrificates of patients who had died from coronary disease

The Departments of Pathology and Forensic Medicine of Turku University took part in the study e.g. by completing the death record forms

Working procedure of the heart registry
The nurse of the Heart Registry visited Turku University Hospital each day and interviewed the patients with myocardial infarction She visited Turku City Hospital every week. Information for register was thus obtained in accordance with recommendations of the WHO In addition special information on patients aged 65 years or more was collected.

A medico-legal post mortem examination was usually performed in cases of sudden death if the patient had not been treated by physician immediately prior to his death. If the patient had attended a physician prior to his death the certificate was filled in by the attending physician without an autopsy

Follow up
A follow-up record containing information about the development, complications and final criteria of the disease was filled e pati discharged from t the days from the ction on, in the case of coronary deaths a death record form was completed disclosing the place of death and death certificate diagnosis the site of infarction and changes in the coronary arteries

The patients were requested to attend the Turku Heart Registry for a check up after a period of three months. This took the form of an interview clinical examination chest roentgenogram (A—P and lateral and ECG including a 3 minute strip from one of the chest leads at a speed of 5 mm/sec to detect extrasystoles and other arrhythmias)

The next follow up took place after one year and consisted of the same examinations.

3 MATERIAL OF PRESENT STUDY

The present study was started on 1 3 1972 and lasted 14 months until 30.4. 1973 During this period a total of 467 patients of 65 years of age or older were registered In this group were 404 cases of myocardial infarction and these cases form the patient material of the present study

4 CONTROL MATERIAL

When the Heart Registry had been in operation for a few months more than 100 patients over 65 years old with myocardial infarction had been discovered. At this stage age and sex matched control persons were selected by computer from the population register of Turku. Because the information in the computer records was valid for the date 1 1 1972 a few controls were discovered to have died These persons (7 men and 9 women) were replaced by new controls chosen randomly from the different parishes of Turku. Each new control was of the same sex and born in the same year as the control patient who had died, and still alive according to the parish files.

All the 127 control patients were asked to attend for a medical check up Of those who attended 38 were men and 62 were women 15 men and 14 women failed to attend. The combined attendance percentage was 79 / (73 / for men and 83 / for women) The subjects were requested to fast for 12 hours prior to their check up i e beginning from the previous evening. On the morning of the check up the following tests were performed serum cholesterol serum triglycerides Haemoglobin ESR,

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If a control patient failed to attend a second request to attend for a check-up was sent, and in case of this being unsuccessfull the patient was rejected from the study.

The author examined each of the control patients clinically. The interview was carried out by the same nurse of the Heart Registry who had previously interviewed all of the patients with myocardial infarction.

5 ADDITIONAL CONTROL MATERIAL

The Turku Heart Registry studied the presenting symptoms in younger patients with myocardial infarction. The symptoms of patients under 65 years old were obtained from the computer records for exactly the same period as that of the time of study (1.3. 1972—30.4. 1973). This was performed in order to compare the presenting symptoms of myocardial infarction in the elderly with symptoms in the younger patients.

6 DIAGNOSTIC CRITERIA

The final diagnosis was made according to the criteria given by the WHO (Working Group of Ischaemic Heart Disease Registers: EURO 8201 (5) 1971).

The diagnosis was based on a history of chest pain, ECG or enzyme changes and if possible, by post mortem examination. The final diagnostic categories were 1. definite 2. possible 3. no infarction, 4. insufficient information (insuff. data). The last mentioned referred on to deaths in which a post-mortem examination was not performed and from whom insufficient information was available.

The history of chest pain was usually taken by interview or sometimes from the hospital records. The definition of pain was the same as that used by the working group of WHO i.e. chest pain lasting for more than 20 minutes and resistant to nitroglycerine. Pain is usually severe and in some cases untolerable.

An effort was made to obtain an ECG in the hospital from the patients with suspected infarction on at least three consecutive days following the attack of pain and also, if necessary later. All the ECG recordings were available in the case reports.

An attempt was made to determine the enzyme levels on at least three consecutive days following the suspected infarction and to repeat these later if indicated. SGOT and LDH estimations were available in all hospitals and in Turku University Central Hospital isoenzyme estimations were also available. The enzymes were considered to be elevated if SGOT level was 30 U/l or more and 19—29 U/l was border line. During the course of investigation there was a change in the laboratory procedure of the normal values on 1.2. 1973. The normal level of SGOT changed from less than 19 U/l to less than 35 U/l and LDH changed from less than 240 U/l to 160—360 U/l.

The WHO's criteria for ECG changes were used (Working Group of Ischaemic Heart Disease Registers: EURO 8201 (5) 1971).

7 DEFINITIONS

Typical chest pain was defined according to the definition of the WHO (EURO 8201 (5) 1971). Cases of definite infarction comprised those patients with the typical diagnostic criteria of definite infarction. Cases of possible infarction comprised those patients in whom the criteria for definite infarction were not fullfilled but in whom the information or data were sufficient for a diagnosis of possible infarction.

Myocardial infarction
In this study acute myocardial infarction was defined as that condition which fulfilled the WHO's criteria of chest pain, ECG and enzyme changes. Also included were those cases with definite signs of myocardial necrosis at post mortem examination.

Angina pectoris
Angina pectoris was defined as chest pain on exertion as it has been earlier defined by the WHO (Arterial Hypertension and Ischaemic Heart Disease 1962).

Hypertension
If hypertension had been discovered before the onset of infarction, this was accepted as a positive finding, either confirmed or unconfirmed depending on the documentation. If hypertension was noticed only after the onset of the infarction it was disregarded.

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Follow up
A follow up record containing information about the development, complications and final diagnostic criteria of the disease was filled in when the patient was discharged from hospital or at the latest 28 days from the onset of the infarction. In addition in the case of coronary deaths, a death record form was completed disclosing the place of death and death certificate diagnosis the site of infarction and changes in the coronary arteries.

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In this study acute myocardial infarction was defined as that condition which *fulfilled* the WHO's criteria of chest pain, ECG and enzyme changes. Also included were those cases with definite signs of myocardial necrosis at post-mortem *examination*.

Angina pectoris
Angina pectoris was defined as chest pain on exertion as it has been earlier defined by the WHO (Arterial Hypertension and Ischaemic Heart Disease 1962)

Hypertension
If hypertension had been discovered before the onset of infarction, this was accepted as a *positive finding*, either confirmed or unconfirmed depending on the documentation. If hypertension was noticed only after the onset of the infarction, it was *disregarded*.

sudden death. In accordance with the directions of the WHO any patient with suspected myocardial infarction or sudden coronary death should be recorded in the register

A clinical and epidemiological study dealing with coronary disease in the elderly began on 1 3 1972 and lasted 14 months until 30.4. 1973

The personel consisted of one nurse and three part time physicians The registry had its own office in the Tuberculosisbureau, where roentgenograms and record forms were filled and the follow up study was performed

All the hospitals and physicians in Turku were notified of the commencement of operation of the heart register The physicians were requested to report patients with myocardial infarction to the registry Circulars were sent and personal visits made to the relevant departments of both Turku University Hospital and Turku City Hospital Paimio Hospital was also notified The cases of sudden coronary death were recorded from the death certificates collected by the city health officer The Turku Heart Registry therefore checked all the death cetrificates of patients who had died from coronary disease

The Departments of Pathology and Forensic Medicine of Turku University took part in the study e g. by completing the death record forms

Working procedure of the heart registry
The nurse of the Heart Registry visited Turku University Hospital each day and interviewed the patients with myocardial infarction. She visited Turku City Hospital every week. Information for register was thus obtained in accordance with recommendations of the WHO In addition special information on patients aged 65 years or more was collected.

A medico legal post mortem examination was usually performed in cases of sudden death if the patient had not been treated by physician immediately prior to his death. If the patient had attended a physician prior to his death the certificate was filled in by the attending physician without an autopsy

Follow-up
A follow up record containing information about the development, complications and final diagnostic criteria of the disease was filled in when the patient was discharged from hospital or at the latest 28 days from the onset of the infarction. In addition, in the case of coronary deaths a death record form was completed disclosing the place of death and death certificate diagnosis the site of infarction and changes in the coronary arteries.

The patients were requested to attend the Turku Heart Registry for a check up after a period of three months. This took the form of an interview clinical examination, chest roentgenogram (A—P and lateral and ECG including a 3 minute strip from one of the chest leads at a speed of 5 mm/sec to detect extrasystoles and other arrhythmias)

The next follow up took place after one year and consisted of the same examinations.

3 MATERIAL OF PRESENT STUDY

The present study was started on 1 3 1972 and lasted 14 months until 30.4 1973 During this period a total of 467 patients of 65 years of age or older were registered. In this group were 404 cases of myocardial infarction and these cases form the patient material of the present study

4 CONTROL MATERIAL

When the Heart Registry had been in operation for a few months more than 100 patients over 65 years old with myocardial infarction had been discovered. At this stage age and sex matched control persons were selected by computer from the population register of Turku. Because the information in the computer records was valid for the date 1 1 1972 a few controls were discovered to have died. These persons (7 men and 9 women) were replaced by new controls chosen randomly from the different parishes of Turku. Each new control was of the same sex and born in the same year as the control patient who had died and still alive according to the parish files.

All the 127 control patients were asked to attend for a medical check up Of those who attended 38 were men and 62 were women 15 men and 14 women failed to attend. The combined attendance percentage was 79 % (73 % for men and 83 % for women) The subjects were requested to fast for 12 hours prior to their check up i.e. beginning from the previous evening. On the morning of the check up the following tests were performed serum cholesterol serum triglycerides Haemoglobin ESR,

chest roentgenogram and ECG (including a 3 minute strip from one of the chest leads at a speed of 5 mm/sec.)

If a control patient failed to attend a second request to attend for a check-up was sent, and in case of this being unsuccessfull, the patient was rejected from the study

The author examined each of the control patients clinically The interview was carried out by the same nurse of the Heart Registry who had previously interviewed all of the patients with myocardial infarction

5 ADDITIONAL CONTROL MATERIAL

The Turku Heart Registry studied the presenting symptoms in younger patients with myocardial infarction. The symptoms of patients under 65 years old were obtained from the computer records for exactly the same period as that of the time of study (1 3 1972—30.4. 1973) This was performed in order to compare the presenting symptoms of myocardial infarction in the elderly with symptoms in the younger patients.

6 DIAGNOSTIC CRITERIA

Th final diagnosis was made according to the criteria given by the WHO (Working Group of Ischaemic Heart Disease Registers EURO 820 (5) 97

The diagnosis was based on history of chest pain, ECG or enzyme changes and if possible by post-mortem examination. The final diagnostic categories were definite 2. possible 3 no infarction, 4 insufficient information (insuft. data) The last mentioned referred on to deaths in which post-mortem examination was not performed and from whom insufficient information was available.

The history of chest pain was usually taken by interview or sometimes from the hospital records. The definition of pain was the same as that used by the working group of WHO e. chest pain lasting for more than 20 minutes and resistant to nitroglycerine. Pain is usually severe and in some cases untolerable.

An effort was made to obtain an ECG in the hospital from the patients with suspected infarction on at least three consecutive days following the attack of pain and also, if necessary later All the ECG recordings were available in the case reports.

An attempt was made to determine the enzyme levels on at least three consecutive days following the suspected infarction and to repeat these later if indicated. SGOT and LDH estimations were available in all hospitals and in Turku University Central Hospital isoenzyme estimations were also available. The enzymes were considered to be elevated if SGOT level was 30 U/l or more and 19—29 U/l was border line. During the course of investigation there was a change in the laboratory procedure of the normal values on 1.2. 1973 The normal level of SGOT changed from less than 19 U/l to less than 35 U/l and LDH changed from less than 240 U/l to 160—360 U/l.

The WHO's criteria for ECG changes were used (Working Group of Ischaemic Heart Disease Registers EURO 8201 (5) 1971)

7 DEFINITIONS

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C=Depth. The greatest horizontal depth of te cardiac shadow The calculated cardiac olume was then divided with body surface rea.

Classification of venous congestion was performed as Turner has proposed (Turner et al 972)

9 COMPUTER TREATMENT

All data were punched and treated by Turku City Computer Center

10 MATHEMATICAL TREATMENT

The difference between men and women were calculated separately and also compared to the control group. The standard deviation was also defined. The significance of the results was calculated by the t-test or Chi-square test.

The statistical significance is represented as follows

N.S.D No statistical significant difference	($p>0,05$)
almost significant	($p<0,05$)
significant	($p<0,01$)
highly significant	($p<0,001$)

Other cardiovascular diseases
Other cardiovascular diseases included valvular diseases cardiomyopathias chronic dysrhythmias and pulmonary heart disease

Diabetes mellitus
The diagnosis of diabetes was accepted as definite if the disease was medically documented. Continuous treatment for diabetes also constituted positive evidence. In the case of patients without sufficient evidence of diabetes the diagnosis was regarded as unconfirmed.

Obesity
The height and weight of the patients were taken from the hospital records during the interview or from the relatives of persons who died suddenly The control patients had their height and weight measured at the time of their check up Height and weight measurements were usually performed in the hospitals

The body mass (i e. relative weight) index used was the Quitelet index (weight divided by height squared) (Keys et al 1972)

Smoking habits
The patients were divided into three groups 1) Those who smoked regularly 2) those who had given up smoking, 3) those who had never smoked.

A regular smoker was defined as one who regularly smoked at least one cigarette per day (or and equivalent amount of pipe or cigar tobacco) or who had smoked this quantity for at least the three preceding months before the acute attack.

An ex-smoker was defined as one who had smoked earlier but who had discontinued the habit at least three months before the start of the acute attack. A non-smoker was defined as one who had never smoked the quantity mentioned above The cigarette smokers were also divided according to their daily consumption of cigarettes while the pipe and cigar smokers made up a separate group of their own. Those who smoked both cigarettes and pipe or cigars were included in the cigarette smokers group

Spare time exercise
The patients were divided into four groups. Group 1 The patient made his way around the city and did his shopping on foot. Group 2 Very little exercise in the city e.g. shopping trip to the nearest shop Group 3 Moved abo[ut] indoors at home only Group 4 Bedridden

Patients ability to look after himself
The patients were divided into two grou[ps] Group 1 Patient able to dress wash and e[at] without assistance. Group 2 Patient requir[es] assistance to perform above mentioned task

Residence
The patients were asked whether their res[i]dence was their own or rented or whether th[e] patient lived in an institution, and also abo[ut] the facilities available e.g. toilet plumbin[g] sewage disposal.

Where the patients are living
This described whether the patient lived alon[e] with a spouse children relation or in an insti[tu]tution. If the patient reguired domestic hel[p] this was recorded. In addition an enquiry wa[s] made as to who managed the patient's finan[]cial affairs etc. Dietary habits were also exam[]ined including the use of sugar salt, standar[d] milk fats (also different grades) beer wine an[d] alcohol.

The QRS-axis
The QRS axis was measured from the ECG This was performed by first calculating th[e] sum of the R and S waves in leads I and III Then the voltage sums were placed on the appropriate limb leads in Einthoven's triangle and perpendiculars extended from the distances representing these sums in leads I and III. The frontal QRS-axis was derived from the site on intersection of these perpendiculars.

8 RADIOLOGICAL METHODS

Measurement of heart size or cardiac volume
Calculation of cardiac volume was based on the formula

V = L x B x D x D x K (Keats T E. et al. 1965) The constant (K) will vary with the focus film distances used

150 cm K = 0,39 used with erect films
100 cm K = 0,38 used with recumbent films

L = Long diameter This line extends from the junction of the superior vena cava and right atrium to the cardiac apex.

B = Broad diameter This line extends from the junction of the right atrium and the

diaphragm to a point on the left heart border at the junction of pulmonary artery and left atrial appendage.

C = Depth. The greatest horizontal depth of the cardiac shadow. The calculated cardiac volume was then divided with body surface area.

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IV
RESULTS

1 REGISTERED CASES OF ACUTE MYOCARDIAL INFARCTION

During the period of investigation (1 3 1972—30 4 1973) a total of 404 infarctions occurred in 389 patients aged 65 years or more.

Patients not examined clinically comprised those who died at home or on the way to hospital, in the out-patients' department or in another location, e.g. summer cottage, or at sea. This category consisted of 87 men and 76 women, giving a total of 163 patients (40 3 %)

The diagnosis of infarction was based on post mortem examination in 51 men (58,6 %) and 37 women (46 3 %) In the remainder the diagnosis was obtained from the death certificate filled in by the patient' attending doctor who completed the certificate on the basis of the patient's previous history All of these patients had known history of angina pectoris or myocardial infarction.

24 infarctions were discovered by *clinical examination* in 226 patients. Of these patients 01 were men 7 of whom had had two infarctions giving a total of 08 infarctions in men I the remaining 25 women 6 patients had two infarctions nd one patient three infarctions (tables 1 2 3 and 4.) Infarction was confirmed by post mortem examination in 71 patients (33 men and 38 women) The ratio of the male to female was 0,81 1

The average age of the men was 73,1± 6 5 years and of th women 74,2± 6 7 years. The average age of the control patients was 72 7± 5 5 years for men and 74,1± 6 7 years for women. The average ages of patients and the control persons did not show a statistical significant difference. The oldest patient was 93 years old and the oldest control person was aged 94

The majority of patients examined clinically fulfilled WHO's criteria for definite infarction. (Table 1.)

Age distribution

Figure 1 shows that the control group follows the age of the patients steadily at a slight distance behind and lso that there are no 65 year old patients in the control group since they had already reached the age of 66. Figure shows the age distribution of patients.

Table 5 shows the age distribution of patients who were not examined clinically

Table 2. Pain in myocardial infarction

	Males	Females	Total
typical chestpain	63	77	140
atypical chestpain	9	8	17
no pain	23	34	57
unknown	13	14	17
	108	133	241

Table 1 Distribution of cases with myocardial infarction according to WHO diagnostic category

	All cases			Clinically examined cases		
	Males	Females	Total	Males	Females	Total
definit myocardial infarction	152	150	302	101	113	214
possible myocardial infarction	7	20	27	7	20	27
insuff. data	36	39	74			
Total	195	209	404	108	133	241

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Table 3 ECG changes after myocardial infarction

	Males	Females	Total
unequivocal	66	48	114
equivocal	21	59	80
other ECG abnormalities	8	16	24
normal ECG	3	1	4
unknown	10	9	19
	108	133	241

Table 5 Age distribution of patients who w[...] examined clinically mostly dying suddenly

Age	Males	Females
65—69 years	30	18
70—74	23	20
75—79	23	16
80—84	7	13
85—89	4	8
90—94	0	1
Total	87	76

Table 4 Serum enzymes after myocardial infarction

	Males	Females	Total
elevated	74	85	159
borderline case	5	7	12
normal	7	22	29
unknown	22	17	39
	108	133	241

Incidence of myocardial infarction in total population of Turku

Table 6 shows that the incidence almost linearly increases in patients from after the age of 40 up to the age of 80—89 years After this a decrease occurs During the period of ... the youngest male patient with myocardial infarction was 26 years old. Another was 27 y... old. The incidence curve of women follows about 10 years behind the incidence curve ... men parallely There was no registered case ... infarction in female patients under the age ... 50 years Figure 2

Comments

In comparison to the incidence in the North Karelia Project (Puska et al. 1974) it can be observed that in Turku the incidence in the 60—69 year age group in both men and women is twice as low as in North Karelia After the age of 70 the incidence in Turku

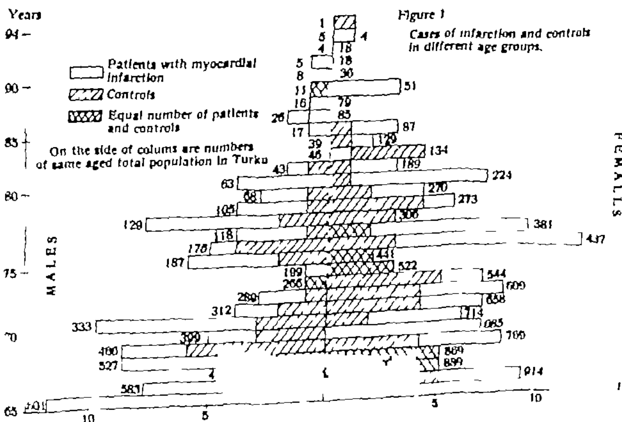


Figure 1

Cases of infarction and controls in different age groups.

Table 6. The incidence of myocardial infarction in total population of Turku

Age	Cases of infarction during 1.3.—1972—28.2.1973		Incidence /1000/ year	
	Males	Females	Males	Females
20—29 v	2	0	0.12	0
30—39 v	4	0	0,38	0
40—49 v	23	0	2,4	0
50—59 v	77	14	10.4	1.4
60—64	19	30	15,0	5.7
65—69 v	62	52	23,6	11,6
70—74	37	51	23,3	15.4
75—79 v	32	43	39.6	20,6
80—84 v	20	30	61,8	27,5
85—89 v	6	12	57.1	28,1
90—94 v	1	1	43,5	12,0

increases considerably. The peak incidence in men in the 80—89 year age group is 60,4 per 1000 per year and the corresponding incidence in women is 33.7 per 000 per year. In Oxford, Kinlen fo nd the incidence in 60—69 year old men to be 13 per 1000 per year while in Tur ku the incidence was 18,4 per 000 per year. The incidence in women there was 3.9 per 000 per year while in Turku the incidence was more than twice this figure (9,6 per 1000 per year).

The incidence in North Karelia appears to be particularly high in younger patients whereas in Turku the older age groups have a high incidence of myocardial infarction.

Kinlen et al. (1973) observed that in Oxford the incidence in 60—69 year old men was 13 per 1000 per year and in women 3.9 per 1000 year. The incidence of women in Turku was twice as high but the incidence of men in Turku was the same as in Oxford.

Medalie (1973) found that in Israel the over all incidence in men over the ge of 65 years was 20,4 per 1000 per year a figure which is clearly lower than in Turku.

Summary

The incidence is highest in men in the age group of 80—84 years (61,8 per 1000 per year). The incidence is highest in women in the age group of 85—89 years (28.1 per 1000 per year). The incidence curve of women follows about 10 years behind the incidence curve of men parallelly.

Monthly variation

There were no statistically significant variation in the incidence and mortality in different months. The incidence is highest in women in March and May and lowest in June and August. The incidence in men is distributed more

Figure 2. The incidence curv 1000 year

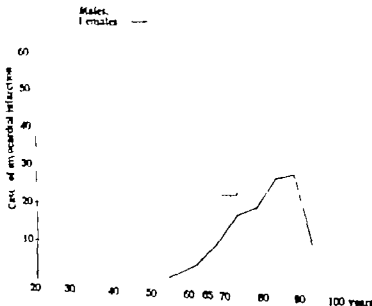


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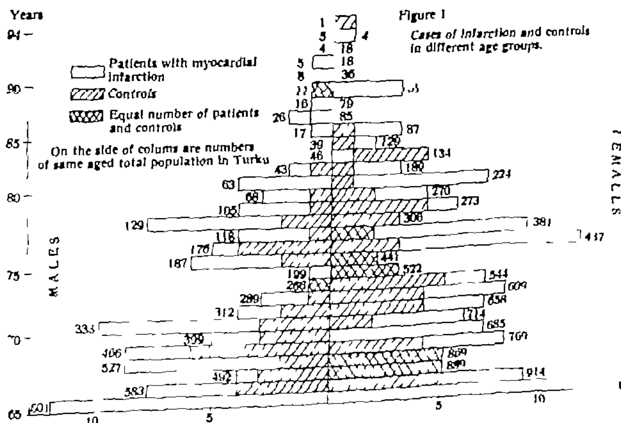


Figure 1
Cases of infarction and controls in different age groups.

xamined. It was noticed that most infarctions ccurred between th hours of 7 m. and midday (17 men and 21 women, giving a total of 38 patients) Correspondingly the least number of infarctions occurred between the hours 1 p.m. and 6 p.m. (14 men and 11 women giving a total of 25 patients) In addition between the hours 7 p.m. and midnight there was a total of 34 cases of infarction (16 men and 8 women) Nevertheless in many instances the exact time could not be deter mined, partly due to the vague nature of the symptoms of infarction and partly because of th poor memory of the patients or dementia. T ble 8.

Table 8. Time of day of the onset of myocardial infarction

Time	Males	Females	Total
0 – 6 a.m.	14	16	30
6 – 12 a.m.	17	21	38
0 – 6 p.m.	14	11	25
6 – 12 p.m.	16	18	34

Comments
Hagfeld (197) found in Copenhagen that most infarctions occurred between the hours 4 p.m. and midnight. In Finland Vartio (960) also noticed that in the case of hospital patients th majority of infarctions occurred at night during the time of rest (34,6 /)

Summary
During the period of 24 hours no statistically significant difference was found in the occurr ence of infarctions

Mortality
The total number of patients with myocardial infarction was 389 (88 men and 20 women)

At home or during the transport to hospital (mostly sudden death) died 87 men (47 3 °/) and 76 women (37,8 /)

In the hospital 40 men died and the cumulative mortality from beginning was 127 men (67 6 /) In hospital 52 women died and the cumulative mortality was 128 women (63 7 /o)

28 days after the infarction the cumulative mortality was 130 men (69 1 /) and 129 women (64,2 /)

After 3 months the cumulative mortality was 34 men (71 3 /) and 135 women (67 7 /o)

After one year the cumulative mortality was 145 men (77 1 /) and 151 women (75,6 /)

Table 5 shows the age distribution of patients who were not examined clinically dying mostly at home.

Mortality in the CCU was in men 17,4 / (4 men from 23 men) and in the ordinary medical ward 48,0 / (36 men died)

Mortality of women in the CCU was 18 9 / (7 women died from 37 women) and in the ordinary medical ward it was 46,9 / (45 women died)

Comments
In Malmo Johansson (972) found hospital mortality rate of 33 1 °/ (30,4 / for men and 38 5 ° for women) The figures apply to the whole material, including young patients. Puska et al. (1974) observed that the hospital mortality in younger patients was appreciably lower in elderly patients. In that study 64 / of the men and 62 / of the women had died after half a yea and 67 / of the men and 63 / of the women after a year (elderly patients)

Table 9. The hospital mortality and survivors in different age groups

Age	number of deaths		mortality /1000, year		survivors /1000/ year		case fatality	
	Males	Females	Males	Females	Males	Females	Males	Females
65 – 69	29	27	10,8	6,0	12,3	5,6	29/63	27/52
70 – 74	29	29	18,1	9,0	5,0	6,6	29/37	29/51
75 – 79 v	24	31	29 7	14,8	9,9	5,7	24/32	31/43
80 – 84	16	20	49,2	18,2	12,3	9 1	16/20	20/30
85 – 89 v	4	9	38,1	20,9	19,0	6,9	4/6	9/12
90 – 94 v	1	1	43,5	12,0	0	0	1/1	1/1

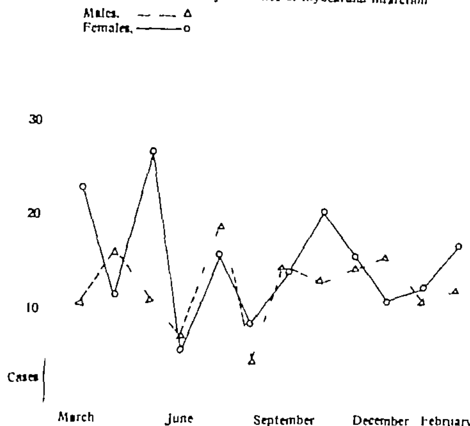


Figure 3 Monthly incidence of myocardial infarction

evenly and it is highest in July and lowest in June. (N.S.D.) Figure 3

The peak of mortality curve is in men in June and in women in May (N.S.D.)

Comments

Protos et al. (1971) noticed two peaks in the incidence of infarction in women one in December and a second in April. The mortality in women was also highest during the spring.

Johansson (1972) found the lowest incidence in August as also was the case in the present study when the total number of infarctions are taken into consideration. In the case of clinically examined patients it was observed that the figure for August was distinctly the lowest in both men and women and also that the figure for June was clearly higher than for August. The mortality rate in August appears to be higher perhaps due to the fact that the patients with infarction did not survive long enough to be investigated in hospital.

Vartio (1960) found that in Oulu the majority of infarctions occurred in February and the least in April. 27.8 % of the infarctions occurred during the winter months and 22,8 % during the spring months. These results are taken from the whole of Vartio's material, including young patients.

Summary

There was no statistically significant variation in the incidence and mortality in different months.

Days of the week

The infarctions were distributed fairly evenly among the different days of the week. There were no statistical differences between the different days of the week (N.S.D.) Table 7

Table 7 Weekday of the onset of myocardial infarction

	Males	*Females*	*Total*
Sunday	7	12	19
Monday	12	11	23
Tuesday	11	8	19
Wednesday	16	11	27
Thursday	11	16	27
Friday	8	16	24
Saturday	10	13	23
unknown	33	46	79

24 hour period

When information about the onset of initial symptoms became available the distribution of infarctions over each 24 hour period was

examined. It was noticed that most infarctions occurred between the hours of 7 a.m. and midday (17 men and 21 women, giving a total of 38 patients). Correspondingly the least number of infarctions occurred between the hours 1 p.m. and 6 p.m. (14 men and 11 women, giving total of 25 patients). In addition between the hours 7 p.m. and midnight there was a total of 34 cases of infarction (16 men and 18 women). Nevertheless in many instances the exact time could not be deter mined partly due to the vague nature of th symptoms of infarction and partly because of the poor memory of the patients or dementia. Table 8

Table 8. Time of day of the onset of myocardial infarction

Time	Males	Females	Total
0–6 a.m.	14	16	30
6–12 a.m.	17	21	38
0–6 p.m.	14	11	25
6–12 p.m.	16	18	34

Comments
Hagfeld (197) found in Copenhagen that most infarctions occurred between the hours 4 p.m. and midnight. In Finland Vartio (1960) also noticed that in the case of hospital patients the majority of infarctions occurred at night during the time of rest (34,6 %).

Summary
During the period of 24 hours no statistically significant difference was found in the occurrence of infarctions.

Mortality
The total number of patients with myocardial infarction was 389 (88 men and 201 women).
At home or during the transport to hospital (mostly sudden death) died 87 men (47,3 %) and 76 women (37,8 %).

In the hospital 40 men died and the cummulative mortality from beginning was 127 men (67,6 %). In hospital 52 women died and the cumulative mortality was 128 women (63,7 %).

28 days after the infarction the cumulative mortality was 130 men (69,1 %) and 129 women (64,2 %).

After 3 months the cumulative mortality was 134 men (71,3 %) and 135 women (67,7 %).

After one year the cumulative mortal ty was 145 men (77,1 %) and 151 women (75,6 %).
Table 5 shows the age distribution of patients who were not examined clinically dying mostly at home.
Mortality in the CCU was in men 17,4 % (4 men from 23 men) and in the ordinary medical ward 48,0 % (36 men died).
Mortality of women in the CCU was 18,9 % (7 women died from 37 women) and in the ordinary medical ward it was 46,9 % (45 women died).

Comments
In Malmö Johansson (1972) found a hospital mortality rate of 33,1 % (30,4 % for men and 38,5 % for women). The figures apply to the whol material including young patients. Puska et al. (1974) observed that the hospital mortality in younger patients was appreciably lower in elderly patients. In that study 64 % of the men and 62 % of the women had died after half a year and 67 % of the men and 63 % of the women after a year (elderly patients).

Table 9. The hospital mortality and survivors in different age groups

Age	number of deaths		mortality /1000/ year		survivors /1000/ year		case f tality	
	Males	Females	Males	Females	Males	Females	Males	Females
65–69	29	27	10,8	6,0	12,3	5,6	[illegible]	[illegible]
70–74	20	20	18,1	9,0	5,0	6,8	[illegible]	[illegible]
75–79	24	31	29,7	14,6	9,9	5	[illegible]	[illegible]
80–84	16	20	40,2	19,2	12,3	8 1	[illegible]	[illegible]
85–89	4	9	38,1	20,9	19,0	6	4	[illegible]
90–94	1	1	43,5	12,0	0	0	[illegible]	

Vartio (1960) noticed in Oulu that pa tients over 65 years old had a hospital morta lity rate of 38,9 %

Cosby et al. (1973) observed that the mortal ity rate rose with increasing age and in the 60—69 year age group it was about 30 % in the 70—79 year age group about 40 % and over 80 years old the rate was about 50 %

Summary

Because in this study the mortality of myocardial infarction in the total popu lation of Turku is studied the numbers are higher than the numbers of other invest Almost 40 % of the clinically examined tients died in hospital and from all pa were over 60 % at that time dead. These res show that myocardial infarction in elderly is grave illness.

Survival rate

101 men and 125 women came to alive. During the period of hospitalisation men (52 7 %) and 73 women (62,2 %) surviv Figure 5

Figure 4 Mortality monthly in clinically examined patients

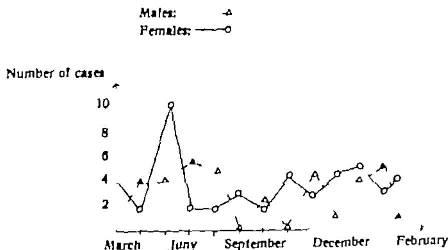
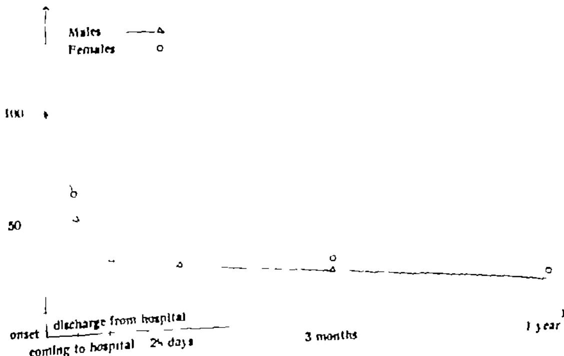


Fig re 5 S rviv rs (%) after onset f myocardial infarction in the t tal populati of Tu ku



28 days after the infarction 58 men (30,9 %) and 72 women (35,8 %) were still alive.

After 3 months 54 men (28,7 %) and 66 women (32,3 %) were living. After a year 43 men (22,9 %) and 50 women (24,4 %) were still alive.

2 ATMOSPHERIC PRESSURE AND TEMPERATURE

Atmospheric pressure at the start of myocardial infarction was investigated in 37 cases of infarction in men (34,3 %) and 45 cases of infarction in women (33,8 %) and it was mostly 750—759 mmHg. According to information received from the Turku Meteorological Office the average atmospherical pressure in 1972 was 76 7 mmHg and in 1973 758,2 mmHg. The atmospheric pressure thus did not appear to have any significance (N.S.D.)

Temperature Both the winter and spring of 1972 and the winter of 1973 were relatively mild. The lowest temperature reached during the period of the investigation was —21 7 C on 26.2.73 The highest temperature was +31,0 C on 9 6 72. The average temperature in 197 was +5,8 C and in 1973 +5 1°C. The majority of th infarctions occured while the temperature was between + C and +9 C (36 in men (33 3 %) and 41 in women (30,8 %)) The relative humidity in 1972 was 81 % and in 1973 80 %. The role of temperature was not found to be of statistical significance.

Comments

Sotaniemi et al. (1970) have observed that variation in temperature above or below th monthly average is significantly associated with an increased incidence of myocardial infarction.

Summary

In this study temperature was found to have no role in the incidence of myocardial infarction.

3 TRANSPORT TO HOSPITAL

Almost half of patients (44 men and 45 women) were transported to hospital by ambulance. Only two men came to hospital on foot.

4 DELAY

In this study the total delay i.e. the time difference between the onset of symptoms and arrival at hospital, was investigated. The median of the delay in men was 55 minutes and in women 75 minutes.

Comments

Puska et al. (1973) noticed 2 peaks in men in North Karelia, one at 60—69 minutes and a second at 2 hours. In women the distribution was similar being divided equally into 3 groups arriving 30—59 minutes 2 hours and 3 hours after the onset of symptoms. Johansson (1972) found an average total delay of 30 9 hours in Malmo.

Summary

Because of the difficulty in the definition of the various components of the delay in the elderly only the total delay was investigated in this study Th delay in Turku was relatively short. Women arrived for treatment relatively more slowly since the median delay was 20 minutes longer

5 FACTORS PRECEDING INFARCTION

Exertion

There was a definite history of exertion or stress preceding infarction in 20 patients. The infarction occurred during this time or immediately following it. The most common form of exertion was heavy physical work e.g. cleaning or gardening. In two men, a visit to the sauna had preceded the infarction. A comparison between these factors and the same factors preceding infarction in younger patients over the same period revealed no statistical difference.

Pre-existing infections

As can be seen from table 10, the most common infections preceding infarction were common cold and bronchitis.

Stroke

15 patients (4 men and 11 women) developed infarction following a stroke. The stroke generally preceded the infarction by 1—2 weeks. However when the cases of stroke

Vartio (1960) noticed in Oulu that patients over 65 years old had a hospital mortality rate of 38 9 %.

Cosby et al (1973) observed that the mortality rate rose with increasing age and in the 60—69 year age group it was about 30 % in the 70—79 year age group about 40 % and over 80 years old the rate was about 50 %.

Summary

Because in this study the mortality of myocardial infarction in the total population of Turku is studied the numbers are higher than the numbers of other investigators. Almost 40 % of the clinically examined patients died in hospital and from all patients were over 60 % at that time dead. These figures show that myocardial infarction in the elderly is grave illness.

Survival rate

101 men and 125 women came to hospital alive During the period of hospitalisation 61 men (52 7 %) and 73 women (62,2 %) survived. Figure 5

Figure 4 Mortality monthly in clinically examined patients

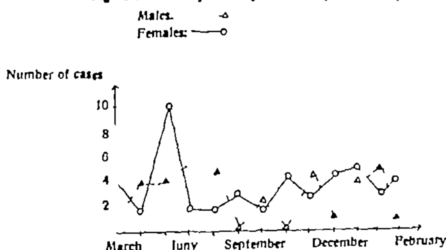
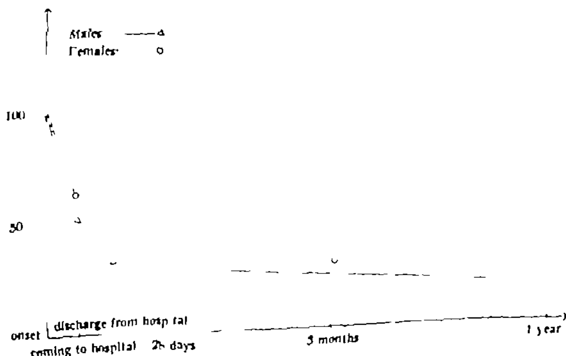


Figure 5 Survivals (%) after onset of myocardial infarction in the total population of Turku



5 patients had a history of car liac failure and one of these had mitral in-competence. Dyspnoea was the presenting symptom in 20 men (18 55) and 29 women (21,8 %) A presenting symptom of severe spnoea was associated with a mortality of 60 / in men (12 patients) and 55,2 / in women (16 patients) There was significantly higher hospital mortality in patients with in-farction presenting with acute dyspnoea com-pared to those presenting with chest pain ($p < 0{,}01$) Acute dyspnoea was also associated with other symptoms In three patients dysp-noea was associated with an episode of loss of consciousness and in three further patients with palpitations, vomiting and confusion re-spectively

In many cases chest pain was associated with sensation of dyspnoea but this symptom was not regarded as true dyspnoea.

The table 6 shows that the majority of pa tients complaining of dyspnoea had history of cardiac failure and diabetes. These were followed by a history of stroke In men, a his-tory of chronic bronchitis and emphysema and myocardial infarction was also common. Ac-cordingly infarction should always be suspect ed if patient with cardiac failure bronchitis or diabetes develops acute dyspnoea

Los of consc ousness

The third most common presenting symptom was an episode of loss of consciousness. The episode generelly lasted bout 5 minutes. A total of 22 patients (9) presented with this symptom This total was made up of 8 men (7,4 %) and 14 women (0,5 /%) and the e-spective hospital mortality for men and women was 75 % (6 patients) and 50 / (7 patients) Tables 1 and 3

It is evident therefore that loss of con sciousness is an ominous presenting symptom nd follows severe infarction

In 6 patients loss of consciousness was the only presenting symptom One of these pa-tients was discovered to have ventricular fib-rillation which was reverted to sinus rhythm by DC shock. A few patients presented with loss of consciousness associated with chest pain. In 2 further patients loss of consciousness associated with myocardial infarction was complicated by the development of stroke.

Loss of consciousness in men was associated with a past history of infarction in 4 cases and a past history of stroke in 3 cases. There was an association with heart failure in 3 cases and diabetes in 2 cases. In women there was an association with heart failure (3 cases) dementia (3 cases) diabetes (2 cases) and past history of stroke (2 cases) In the majority of cases loss of consciousness appeared to be asso-ciated with a history of either heart disease stroke or diabetes. The symptom occured chief ly in elderly women whose average age was 77 years.

Case report

An 8 year old mal retired gardener was admitted to hospital following an episode of loss of conscious-ness. He had had left inguinal hernia repaired 25 years previously and right inguinal hernia repaired 3 years previously Four months prior to admission he had attended doctor because of dyspnoea H was found to hav cardiac enlargement and treat ment was initiated. The patient, however stopped taking his medication after short time as he felt that he had not obtained any real benefit One week prior to admission he had suffered a number of episodes of nausea, vertigo and dyspnoea which were relieved by resting On 14 73 while rushing for taxi, he developed vertigo, nausea and dyspnoea and subsequently loss of consciousness in the taxi There was no history of chest pain. On arrival at the out-patients' department the patient had regained consciousness H denied ny chest pain On exam ination his radial pulse rate was 68/min and was completely irregular (atrial fibrillation) B P 90/60 Cardiac auscultation revealed grade 3 precordial systolic murmur maximal t the apex

ECG on arrival showed ST elevation in lead III and definite, Q-waves in II, III and VF T-waves

Table 13 Presenting symptoms in myocardial infarction and their relationship to hospital mortality

	Male	died		Female	died	
chest pain	72	16	22,2	85	29	34,1
acutedyspnoea	20	12	60,0	29	16	55,2
loss of consciousness	8	6	75,0	14	7	50,0
vertigo and weakness	4	2	50,0	13	5	38,5
ecurrent vomiting	4	2	50,0	13	3	23,1
confusion	3	3	66,7	9	4	44 4
palpitation	2	1	50,0	7	4	57 1
upper abdominal pain	4	3	75,0	1	1	100,0

Table 10 Infections preceding myocardial infarction

	Males	Females	Total
Common cold	3	6	9
Acute bronchitis	1	3	4
Maxillary sinuitis	0	1	1
Pneumonia	0	1	1
Acute gastroenteritis	1	4	5
Pyelonephritis	0	2	2
Acute cholecystitis	0	1	1
Diabetic gangrene and osteitis	0	1	1
	5	19	24

preceding the infarction by a longer interval are taken into account, a total of 21 patients (6 men and 15 women) is obtained

Operation or procedure preceding infarction
Only 7 patients had undergone an operation before developing infarction Infarction was preceded by bronchoscopy in one man and by sternal puncture in one man and one woman. Table 11

Table 11 Operations preceding infarction

	Males	Females	Total
Thigh amputation	1	1	2
Cataract operation	1	0	1
Vein bypass -operation	1	0	1
Implantation of Pacemaker	1	0	1
Cholecystectomy	0	1	1
Fixation of hip fracture	0	1	1
Total	4	3	7

6 PRESENTING SYMPTOMS O MYOCARDIAL INFARCTION AND ASSOCIATED MORTALIT

The presenting symptoms of myocardial in farction in the elderly were found to diff from those in younger patients.

Chest pain
Table 12 shows that chest pain was present i 72 men (66 7 % and 85 women (63 9 % Chest pain was clearly the most common pre senting symptom. The relationship betwee chest pain and hospital mortality was also in vestigated and the results obtained showed tha 16 men (22,2 %) and 29 women (34,8 %) wit this symptom died

Duration of chest pain in infarction
The duration of chest pain was less than 6 hours in most cases (101 patients) (Table 13 In 27 patients with chest pain the duration of pain was not clearly established. Painless in farction was associated with the highest mortal ity (64 9 %) Chest pain lasting less than 6 hours was associated with a hospital mortality of 16 7 % i e the mortality was significantly lower ($P < 0 001$)

Dyspnoea
Dyspnoea was the second most common pre senting symptom occurring in 49 patients (20 3 %) The dyspnoea was of acute onset and severe intensity and the patient often appeared ill. In 31 patients dyspnoea was the only presenting symptom 7 of these patients were suffering from chronic bronchitis and

Table 12. Presenting symptoms in clinically examined patients

241 cases of myocardial infarction 108 men and 133 women

	Males		Females		Total	%
chest pain	72	66 7	85	63,9	157	65 1
acute dyspnoea	20	18,5	29	21,8	40	20,3
loss of consciousness	8	7 4	14	10,5	22	9 1
vertigo and weakness	4	3 7	13	9,8	17	7 1
recurrent vomiting	4	3 7	13	9,8	17	7 1
confusion	3	2,8	9	6 8	12	5,0
sudden death in hospital	5	4,6	6	4,5	11	4 6
palpitations	2	1 9	7	5,3	9	3 7
upper abdominal pain	4	3 7	1	0,8	5	2 1
stroke	0		1		2	
peripheral gangrene	1		1		2	
convulsions and shock	2		0		2	
diarrhoea	1		0		1	
lower abdominal pain	1		0		1	
food stuck in throat	1		0		1	

mphysema, 5 patients had a history of car ·iac failure and one of these had mitral in ompetence Dyspnoea was the presenting ymptom in 20 men (18 55) and 29 women ,8 /o) A presenting symptom of severe yspnoea was associated with a mortality of o / in men (12 patients) and 55,2 / in vomen (16 patients) There was significantly nigher hospital mortality in patients with in arction presenting with acute dyspnoea com pared to those presenting with chest pain (p<0,01) Acute dyspnoea was also associated with other symptoms In three patients dyspnoea was associated with an episode of loss of consciousness and in three further patients w th palpitations vomiting and confusion re spectively

In many cases chest pain was associated with a sensation of dyspnoea but this symptom was not regarded as true dyspnoea.

The table 16 shows that the majority of patients complaining of dyspnoea had a history of cardiac failure and diabetes These were followed by history of stroke In men, a history of chronic bronchitis and emphysema and myocardial infarction was also common Ac ordingly infarction should always be suspect ed if a patient with cardiac failure bronchitis or diabetes develops acute dyspnoea.

Loss of consciousness

The third most common presenting symptom was an episode of loss of consciousness. The episode generally lasted about 5 minutes. A total of 22 patients (9 /) presented with this ymptom This total was made up of 8 men (7,4) and 24 women (1 5 /o) and the re pectiv hospital mortal ty for men and women was 75 (6 patients) and 50 / (7 patients) Tables 1 and 3

It is evident, therefore that loss of con sciousness is an ominous presenting symptom nd follow severe infarction

In 6 patients loss of consciousness was the only presenting symptom. One of these patients was discovered to have ventricular fibrillation which was reverted to sinus rhythm by DC shock. A few patients presented with loss of consciousness associated with chest pain. In 2 further patients loss of consciousness associated with myocardial infarction was complicated by the development of stroke.

Loss of consciousness in men was associated with a past history of infarction in 4 cases and a past history of stroke in 3 cases There was an association with heart failure in 3 cases and diabetes in 2 cases In women there was an association with heart failure (3 cases) dementia (3 cases) diabetes (2 cases) and past history of stroke (2 cases) In the majority of cases loss of consciousness appeared to be associated with a history of either heart disease stroke or diabetes. The symptom occured chiefly in elderly women whose average age was 77 1 years.

Case report

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were peacked in V_1—V_4 and ST-depression in I, II and V_5—V_6. SGOT (26) was normal and LDH (367) slightly elevated. The leucocyte count was 6600 and ESR rose from 26—44. The patient developed a fever on the third day his temperature rising to 37,8 C. A lignocaine drip was started on admission. A chest roentgenogram showed that the heart size was with in the upper limits of normal The central pulmonary vessels were distended and there was a right pleural effusion. The patient was digitalised on 23/1/73. On the day of his discharge on 31/1/73 he collapsed suddenly in the corridor and resuscitation was unsuccesfull.

At post mortem examination the heart weighed 540 g. and there was left ventricular hypertrophy 800 ml of coagulated blood was present in the peri cardial sac. This blood resulted from cardiac rupture and had caused tamponade. There was an extensive area of infarction in the posterior wall of the left ventricle and in the posterior aspect of the septum The infarct was partly in the stage of resorption and in some areas appeared to be more recent. At the apex of the left ventricle a 1 cm long rupture was present. There was marked sclerosis of both branc hes of left coronary artery but no recent trombosis was observed. The right coronary artery was narrow ed at its origin. The mitral valve was within normal limits but the aortic valve orifice would admit only one finger and all the valves cusps were markedly arteriosclerotic. There was severe pulmonary con gestion. The liver showed evidence of mild chronic congestion. Death was caused by cardiac infarction and cardiac rupture.

Confusional state

Confusion occurred as a presenting feature of myocardial infarction in a total of 12 patients. It was present in 3 men (2,8 %) and 2 of these died (66 7 %) Confusion was more common in women occurring in 9 patients (6,8 %) 4 of whom died (44,4 %) Tables 12 and 13

Confusional states were most common in elderly women The average age of patients presenting with confusion was 77,4 years Confusion generally occurred in association with chest pain or dyspnoea. In one case confusion was the only presenting feature and no further symptoms were elicited Confusion associ ated with chest pain occurred in 9 patients In addition one patient had vertigo one had dyspnoea and another had associated vomiting.

Other illnesses in confused patients.

One of the men had emphysema and another had carcinoma of the lung. In a third patient myocardial infarction and stroke occurred about one year previously 6 of the women had heart failure 4 had diabetes and 2 had hypertension.

Confusion thus occurred mainly in patients with heart failure pulmonary disease or dia betes.

Case report

A 73 year-old widow of a sheet-metal wo[rker] was admitted to hospital after her daughter fo[und] her lying on the floor of her home. She had [had] diabetes for a number of years, which was be[ing] treated with oral hypoglycemic drugs. One ye[ar] prior to admission she had had a stroke which le[ft] her with a right-sided weakness. She lived al[one] and her general condition had recently been rath[er] poor In December 1972 the patient developed dr[y] gangrene of her right fourth toe and this toe w[as] subsequently amputated on 2/1/73 in Turku Unive[r]sity Central Hospital. She made a satisfactory re[]covery from the operation.

On addmission to Turku City Hospital the patie[nt] was able to walk unaided. Examination show[ed] evidence of an old right hemiparesis. There was [an] obvious clumsiness of movement and increase[d] muscle tone in the right hand. The pulse was [] min, regular BP 195/90. No heart murmurs we[re] audible. At the amputation site a fistula was pre[s]ent and also necrosis of the skin and cellula[r] proximal to he fistula. The patient was transferr[ed] to a surgical ward for amputation.

A right mid thigh amputation was performed o[n] 2/3/73 because of gangrene. Postoperatively her con[]dition was comfortable. There was considerabl[e] blood loss from the amputation site. The systol[ic] blood pressure dropped to 90—110 mmHg. On th[e] evening following the operation the patient's breath ing became laboured and on 3/3/73 she became con fused and completely disorientated. Deep uncon sciousness followed The patient died peacefully o[n] 4/3/73 at 10 35 a.m. without regaining consciousness

A medico-legal post mortem examination was per formed. The heart weighed 300 g. On the surface of the heart there was a layer of fibrin and reddish granular area, apparently due to pericarditis. The margins on the mitral valve were mildly sclerotic and some calcified plaques were present on the aortic valve The left ventricular muscle was 7 mm thick and in the septum and posterior wall from the apex to the base multiple yellowish and recent areas of infarction were observed. There were also a reddish area in the stage of resorption. At the apex an old scar surrounded by an area in the stage of resorption was present The right ventric was about 2 mm thick The coronary arteries were moderately sclerotic and the right coronary artery was markedly narrowed 3 cm from its origin. In the left coronary artery at 2 cm from its origin there was an intramural thrombosis which obstructed the ves sel. Microscop cally a recent infarct was visible containing polymorphonuclear leucocytes and a clea demarcation line from an anuclear rea of heart muscle An ECG had not been performed and the most recent recording had been taken on 11/1/73 This recording showed left anterior hemi block and there was no evidence of infarction. Serum enzymes had not been measured. The diagnosis of myocardial infarction was not made until post mortem examination. The patient's confusion was apparently due to a fall in blood pressure. On 3—4 3 1973 the patient's blood pressure ranged from 90—110 mm. Hg.

Vertigo and weakness

Vertigo was characterized by true of weakness associated

of everything going black" This was associated with collapse in some cases. Th patients did not complain of actual rotational vertigo. Vertigo occurred in 4 men (3 7 /) and 13 women (9,8 /) making 17 patients in all (7 1 /) Tabl 12. The average age of men complaining of vertigo was 73 5 years and of women 75 7 years. Two men (50) and 5 women (38 5 /) complaining of vertigo died. Table 13

The occurrance of vertigo as the single presenting symptom was rare, being present as uch in only 2 patients. It was frequently associated with vomiting and dyspnoea.

Each of the men with vertigo had a heart disease, two had heart failure one had a previous infarction and one had complete heart block.

Of the women, 6 had heart failure 3 had had a previous myocardial infarction, 5 were diabetics, 5 had hypertension and only one had had a stroke

Vertigo therefore, appeared to be associated with history of heart failure previous myocardial infarction and diabetes

Case report

A 69-year-old retired female needleworker was ad mitted to hospital following an ttack of vertigo I the spring of 972 she had suffered cerebral thrombosis, which was treated in the neurological ward Her main complaint had been dysphasia and she subsequently made satisfactory recovery from this symptom The patient also suffered from hypertension.

The patient had symptoms of coryza at home thmes In addition she had postural vertigo and on the evening of 7 973 she developed an ttack of vertigo and simultaneous headache She also complained of seeing «yellow balls» before her eyes At he out patient' department her condition had already improved to the extent that she was entirel asymptomatic and did not complain of chest pain On examination, her general condition ppeared to be satisfactory The blood pressure in her left arm was 5/95 sitting There was no murmur audible on heart auscultation The lungs were clinically clear ECG on arrival showed in erted T-waves in and VF and also in V₂—V₃. Q-waves were present in 1 and VF On the following da occasional right ventricular extrasystoles were observed Chest roentgenogram showed left ventricular enlargement, but the pulmonary vessels were within normal limits The patient was also found to have maxillary sinuitis

Investigations included Hb 8 g/l, W C C 3 000 SGOT 94 U/l and LDH 6 7 U/l. During her hospitalisation the patient remained alert, active and asymptomatic and did not complain of chest pain On 1 973 she developed chest pain, became cyanotic and nconscious and died at o p m on the evening of 973

A post-mortem examination obstruction of the right coronary artery w th recent thrombus was seen this having caused fairly vast infarct in the w ll of the right ventricle. Mural thrombus was present in the right ventricle nd right auricle. In addition, embolisation had occured in the branches of the pulmonary artery mainly in the branches of the left pulmonary artery and in the branch to the right middle lobe. The immediate cause of death was thought to be pulmonary embolism secundary to coronary thrombosis.

Recurrent nausea and vomiting

Vomiting was associated with myocardial infarction in 17 patients (7 1 /) 4 (3 7 /) of total were men and 2 of these died (50 /) Of the 13 women (9,8 /) with this symptom 3 died (23 /) Tables 12 and 13 Patients who vomited after receiving an analgesic were not included. A review of these patients' previous illnesses did not reveal any common factor.

Vomiting was often present in association with dyspnoea or chest pain. Vomiting was the only symptom in one patient, but in this case there was a history of chronic pyelonephritis and uraemia.

Case report

An 81 year old etired cleaning woman was admitted to hospital with persistent vomiting. The patient, who lived in home for the aged, had a past history of rheumatoid arthritis. Prior to admission she developed a urinary tract infection which was treated with tetracycline. Immediately after this treatment was initiated, the patient began omiting The vomiting continued for hours and because her general condition deteriorated she was transferred to Turku University Central Hospital

On arrival, the patient looked ill. She was vomiting and appeared to be severally dehydrated. Blood pressure was 110/60 600 ml of 7,5 % sodium bicarbonate solution was given intravenously immediately and the patient also received normal saline and glucose An Astrup performed on admission showed ph 6,9 pCO_2 2,5 $p\,HCO_3$ less tha 6 base excess 22 Serum creatinine was 336 umol/l

An ECG performed on arrival showed deep Q-wave in lead 1, ST-elev tion in VR and ST depression in leads 1 VL and V₄—V There were tall, peaked T-waves in V₁—V The patient had evidence of urinary tract infection. Blood sugar on admission was 14,9 mmol/l

The patient was admitted on 1 197 and died on the following day At post mortem examination bronchopneumonia was discovered. The heart weighed 300 g. and showed evidence of recent infarction in the anterior wall and septum The coronary arteries were sclerotic and the left circumflex branch was stenosed about 4 cm. from its origin, but no definite thrombosis was found. The patient thus had definite recent myocardial infarction. She had not complained of chest pain t any stage

Palpitations

A total of 9 patients (3 7) had a presenting complaint of palpitation. The total was made

were peacked in V_1—V_4 and ST-depression in I II and V_5—V_6. SGOT (26) was normal and LDH (367) slightly elevated. The leucocyte count was 6600 and ESR rose from 26—44. The patient developed a fever on the third day his temperature rising to 37,8 C. A lignocaine drip was started on admission. A chest roentgenogram showed that the heart size was with in the upper limits of normal. The central pulmonary vessels were distended and there was a right pleural effusion. The patient was digitalised on 23/1/73. On the day of his discharge on 31/1/73 he collapsed suddenly in the corridor and resuscitation was unsuccesfull.

At post mortem examination the heart weighed 540 g. and there was left ventricular hypertrophy 800 ml of coagulated blood was present in the peri cardial sac. This blood resulted from cardiac rupture and had caused tamponade. There was an extensive area of infarction in the posterior wall of the left ventricle and in the posterior aspect of the septum The infarct was partly in the stage of resorption and in some areas appeared to be more recent. At th apex of the left ventricle a 1 cm long rupture was present. There was marked sclerosis of both branc hes of left coronary artery but no recent trombosis was observed. The right coronary artery was narrow ed at its origin. The mitral valve was within normal limits but the aortic valve orifice would admit only one finger and all the valves cusps were markedl arteriosclerotic. There was severe pulmonary con gestion. The liver showed evidence of mild chronic congestion. Death was caused by cardiac infarction and cardiac rupture.

Confusional state

Confusion occurred as a presenting feature of myocardial infarction in a total of 12 patients. It was present in 3 men (2,8 %) and 2 of these died (66 7 %) Confusion was more common in women, occurring in 9 patients (6,8 %) 4 of whom died (44,4 %) Tables 12 and 13

Confusional states were most common in elderly women. The average age of patients presenting with confusion was 77,4 years. Con fusion generally occurred in association with chest pain or dyspnoea. *In one case confusion* was the only presenting feature and no fur ther symptoms were elicited Confusion associ ated with chest pain occurred in 9 patients. In addition one patient had vertigo one had dyspnoea and another had associated vomiting.

Other illnesses in confused patients

One of the men had emphysema and another had carcinoma of the lung In a third patient myocardial infarction and stroke occurred about one year previously 6 of the women had heart failure 4 had diabetes and 2 had hypertension.

Confusion thus occurred mainly in patients with heart failure pulmonary disease or dia betes.

Case report

A 73 year-old widow of a sheet metal worker was admitted to hospital after her daughter found her lying on the floor of her home. *She had had* diabetes for a number of years which was being treated with oral hypoglycemic drugs. One year prior to admission she had had a stroke which left her with a right-sided weakness. She lived alone and her general condition had recently been rather poor. In December 1972 the patient developed slight gangrene of her right fourth toe and this toe was subsequently amputated on 2/1/73 in Turku Univer sity Central Hospital. She made *a satisfactory re*covery from the operation.

On addmission to Turku City Hospital the patient was able to walk unaided. Examination showed evidence of an old right hemiparesis. There was an obvious clumsiness of movement and increase in muscle tone in the right hand The pulse was 84 min regular B P 195/90. No heart murmurs were audible. At the amputation site a fistula was present and also necrosis of the skin and cellulitis proximal to he fistula. The patient was transferred to a surgical ward for amputation.

A right mid-thigh amputation was performed on 2/3/73 because of gangrene. Postoperatively her con dition was comfortable. There was considerable blood loss from the amputation site. The systolic blood pressure dropped to 90—110 mmHg. On the evening following the operation the patient's breath ing became laboured and on 3/3/73 she became con fused and completely disorientated. Deep unconsciousness followed The patient died peacefully on 4/3/73 at 10.35 a.m. without regaining consciusness.

A medico-legal post mortem examination was per formed. *The heart weighed 300 g. On the surface of* the heart there was a layer of fibrin and reddish granular area, apparently due to pericarditis. The margins on the mitral valve were mildly sclerotic and some calcified plaques were present on the aortic valve. The left ventricular muscle was 7 mm. thick and in the septum and posterior wall from the apex to the base multiple yellowish and recent areas of infarction were observed. There were also a *reddish area in the stage of resorption.* At the apex an old scar surrounded by an area in the stage of resorption was present The right ventric was about 2 mm. thick. The coronary arteries were mod erately sclerotic, and the right coronary artery was markedly narrowed 3 cm. from its origin. In the left coronary artery at 2 cm from its origin there was an intramural thrombosis which obstructed the ves sel. Microscopically a recent infarct was visible containing polymorphonuclea leucocytes and a clear demarcation line from an an clear area of heart muscle An ECG had not been performed and the most recent recording had been taken on 11/1/73. This recording showed left anterior hemi block and there was no evidence of infarction. Se rum enzymes had not been mea ured The diagosis f myocardial infarction was not made until post mortem exam nation. The patient' confusi n wa apparently due to a fall in blood pressure On 3—4. 3 1973 the patient's blood pressure anged from 90—110 mm. Hg.

Vertigo and weakness

Vertigo was *characterized* by a vague feeling of weakness associated with a visual sensation

able 16. Presenting symptoms in patients less than 5 years old with myocardial infarction in Turku Heart Register during 1.3.1972—30.4.1973

	Males	Females	Total	
chest pain	158	39	197	77,3
oss of consciousness	12	2	14	5,6
ertigo, weakness	10	4	14	5,6
yspnoea	4	3	7	2,7
alpitations	2	0	2	0,8
pain	0	1	1	0,4
ther symptom	13	3	16	6,3
nknown	2	2	4	
	201	54	255	

number of different presenting symptoms ($p < 0{,}001$). Myocardial infarction thus presents with atypical symptoms more often in the lderly than in younger patients.

In the Framingham Study Kannel et al. (970) found that 22 / of the men and 30 / the women entirely unaware of the fact hat they had a myocardial infarction. These patients apparently had had completely painless, silent infarctions. The authors found that iabetes and hypertension were associated with nt myocardial infarction.

Wolperts' Study of 1000 patients in New York (Wolpert 1971) included 12 cases of myocardial infarction in psychiatric patients over 65 years old. 1 of these infarctions were y silent. There was only one case of yocardial infarction in women over 65 years old and this case also remained undiagnosed. The majority of these psychiatric patients were suffering from schizophrenia. Obviously some the cases of silent infarction resulted from the fact that psychiatric patients are unable to relate thei symptoms.

Vartio (1960) found in Oulu that chest pain was also the most common presenting in patients over 65 years old. Dyspnoea occurred n 18 and shock in 32,2 .

In England, Pathy (967) noticed that the most common presenting symptom was dyspnoea nd next came chest pain. He reported 60 incidence of painless myocardial infarction.

Chest pain was discovered to be the most common symptom by Johansson (1973) in Malmö, occurring in 66,9 / of men and 55 6 / of women. Dyspnoea occured in 12,8 of men and 16 5 / of women and vomiting was present in 11 5 of men and 14,9 % of women.

An association between diabetes and painless infarction has been observed by Kuusisto et al (1960). In their series painless myocardial infarction occurred in 11 5 / of the men and 13 1 / of the women.

Rodstein (1956) examined 51 cases of myocardial infarction occurring in elderly patients and found that only 15 patients had a typical clinical course; 21 cases were atypical and 16 infarctions were completely silent.

Librach et al. (1976) found that the chest pain was absent at the onset of infarction in more than one third of the patients. They found that the 30 day mortality in infarction was lower when it started with pain alone than when it started with pain and other symptoms.

Summary

The presenting symptoms of myocardial infarction in the elderly differ significantly from those in younger patients. In this study chest pain was the most common presenting symptom. The next most common symptoms were dyspnoea loss of consciousness, and vertigo. Chest pain is not such a serious initial symptom as, for example acute dyspnoea. Painless myocardial infarction is associated with a greater hospital mortality than infarction presenting with typical chest pain.

7 TREATMENT CENTRES

The majority of cases (163 patients i.e. 67,6 %) were treated in Turku University Central Hospital. 64 patients (26 9 %) received treatment in Turku City Hospital and 2 patients (0 9 %) in Paimio Hospital.

8 CLINICAL CONDITION AT TIME OF INITIAL EXAMINATION

Heart failure was found in 27 men (25 /) and 42 women (31,4 /) on arrival, making a total of 69 patients (28,2 /). Three men and one woman were in a state of shock on arrival (i.e. 4 patients or 1 7 %). In addition, one man and one woman had both heart failure and shock on arrival (i.e. 2 patients or 0,8 /).

9 ECG ON ADMISSION TO HOSPITAL

Arrhythmias

Extrasystolia was the most common arrhythmia. 20 men (18 5 %) and 18 women (13 5 /)

Table 14 Duration of chest pain and its associated hospital mortality (included later occurring chest pain)

	Males	Females	Total	Died	%
chest pain less than 6 hours	44	57	101	17	16,8
chest pain 6—12 hours	14	17	31	11	35,5
chest pain over 12 hours	13	11	24	5	20,8
chest pain of unknown duration	14	14	28	11	39,3
Total	85	99	184	44	23 9
no chest pain	23	34	57	37	64 9*

up of 2 men (1 9 %) one of whom died and 7 women of whom 4 died (57 1 %) Tables 12 and 13 Atrial fibrillation was present in 4 patients on admission. Further two patients had ventricular extrasystoles and 3 patients had sinus tachycardia on admission

The majority of patients with a history of atrial fibrillation did not complain of palpitations. There was a history of atrial fibrillation in 4 men and 6 women. 11 men and 9 women developed atrial fibrillation as a complication of myocardial infarction.

Stroke
Two women presented with stroke in association with myocardial infarction. Neither had atrial fibrillation

Sudden death in hospital
5 men (4,6 %) and 6 women (4,5 %) died suddenly in hospital. These patients had been admitted for investigation of conditions other than myocardial infarction. A review of these patients past histories showed that diabetes was the most common previous illness being present in a total of 6 patients (3 men and 3 women) Two patients (one man and one women) had a previous stroke Two patients had dementia and one patient had not had any known previous illness Table 12

Other symptoms
Upper abdominal pain present in 4 men and 1 woman was the most common of other symptoms. The woman had had a recent cholecystectomy performed. One man who complained only of upper abdominal pain developed mesenteric thrombosis in association with myocardial infarction. Table 12 3 men (75 %) and one woman (100 %) died. Table 13 One man had peripheral gangrene and another had diarrhoea. Two men had convulsions and were shocked on admission. One man complained of lower abdominal pain. Myocardial infarction occured in another patient following chest pain. The chest pain was preceded by a fit of coughing and attemps at throat-clering after the patient felt food stick in his throat.

Painless infarction
There were 57 (23 6 %) cases of completely painless infarction. The majority presented with acute dyspnoea (32 patients) 4 patients had vertigo and 1 patient was confused. A combination of dyspnoea and confusion occurred in 2 patients. Painless myocardial infarction presented with episode of loss of consciousness in 13 patients 3 patients complained of vomiting only

Painless myocardial infarction occurred most often in patients suffering from heart failure and diabetes. There was also an association with chronic bronchitis in men

37 (64 9 %) of the 57 patients with painless myocardial infarction died in hospital. Patients with painless infartcion had a significantly greater hospital mortality rate than those presenting with chest pain ($p<0,001$) Table 14.

Table 15. Associated illnesses in patients presenting with acute dyspnoea

	Males	Females	Total
heart failure	2	10	12
atrial fibrillation	2	2	4
chronic bronchitis and emphysema	5	0	5
diabetes	4	8	12
dementia			
stroke	4	3	7
previous myocardial infarction	4	0	4

Comments
Table 16 shows the presenting symptoms in patients less than 65 years old with myocardial infarction during the period 1 3 1972—30 4 1973 It can be seen that chest pain is significantly more common ($p<0,001$) in younger patients. Elderly patients have a significantly greater

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Comments

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fascicular block on admission (Table 20) AHB was present in 8 patients (3,3 %) and PHB in 4 patients (1,7 %).

The average pulse rate was 81,7 ± 21,7 in men and 86,1 ± 21,1 in women. The systolic blood pressure on admission was 158,1 ± 32,4 mmHg in men and 160,2 ± 35,0 mmHg in women. The diastolic blood pressure on admission (Ds) was 91,3 ± 14,2 mmHg in men and [illegible]1,1 ± 14,3 mmHg in women.

10 MEDICATION ADMINISTERED AT OUT PATIENT DEPARTMENT

32 patients (34 men, 98 women) received no [illegible] at the out-patient department. The time spent at the out-patient department was generally brief and the patients were rapidly transferred to the ward.

35 men (32,4 %) and 34 women (25 %) received analgesics at the out-patient ward making 69 patients altogether (26,6 %). 5 men and 1 woman received medication for arrhythmias. Digitalis and diuretics were given to 5 men and 8 women. 55 men and 56 women (1 1 patients) received various medications at the out-patient department.

11 LABORATORY TESTS PERFORMED AFTER MYOCARDIAL INFARCTION

E.S.R. was performed on several occasions either daily or on every second day. The maximum E.S.R. was generally reached within a week. The average maximum was 55,5 ± 28,2 mm in men and 48,9 ± 27,7 mm in women.

Haemoglobin averaged 136,1 ± 5,2 g/l in men and 132,2 ± 5,1 g/l in women (N.S.D.). The haemoglobin level was less than 120 g/l in 10 men (9,2 %), 7 (7 %) of whom died in the hospital, and in 22 women (13,5 %) of whom 4 died (72,7 %). Anaemia thus appears to be a sign of poor prognosis in patients with myocardial infarction.

Haemoglobin level 120—155 g/l: 73 men (67,4 %) of whom 25 died (35 %) and 95 women (71,4 %) of whom 27 died (28,8 %) had haemoglobin of this level.

Haemoglobin level over 155 g/l was present in 6 men (5,5 %) of whom only one died and 7 women (5,2 %) of whom 2 died (28,8 %).

SGOT levels were followed daily and the maximum figure was generally reached within first three days. The highest level of SGOT was an average 127,5 ± 188,0 U/l in men and 101,6 ± 152,0 U/l in women (N.S.D.).

SLDH was measured after the second day and followed for a week. The average maximum in men was 914,4 ± 790,9 U/l and in women 715,4 ± 699,1 U/L (N.S.D.).

QRS-axis in the ECG on admission was +16,7 in men and +16,1 in women (N.S.D.). The axis on discharge from hospital within 2 weeks was +6,8 in men and 13,1 in women.

12 TREATMENT WARDS

Coronary care units: 23 men and 37 women were treated in the CCU. Male patients were treated in the CCU for an average of 8,8 ± 6,9 day and women for 3,7 ± 2,0 days ($p < 0{,}001$). 4 men (15,7 %) and 7 women (18,9 %) died in the CCU.

Other patients were treated in the ordinary *medical ward*. The early ambulation principle has in general been followed in Turku for some years (Brummer et al. 1956). The average duration of treatment in men was 12,1 ± 11,3 days and in women 13,8 ± 9,3 days.

36 men (33,3 %) and 45 women (33,8 %) died on the medical ward, making a total of 8[illegible] patients (33,6 %).

13 DURATION OF BED REST

Duration of bed rest was in general about 5 days. This approach is based on the short bed rest principle (Brummer et al. 1956). The average duration of bed rest in men was 5,5 ± 6,9 days and in women 4,8 ± 4,1 days ($p < 0{,}01$).

After this period the patients were allowed to sit in bed or in a chair but were not permitted to walk yet. This stage lasted an average of 3,6 days (± 2,8 days) in men and 3,2 ± 3,6 days in women.

Walking around the bed and then gradually for longer periods was started after about 8 days (men 8,7 ± 4,9 days and women 8,0 ± 5,0 days N.S.D.).

Table 17 Extrasystoles on admission to hospital

	Males	Females	Tot
none	63	92	1.
multifocal ventricular extrasystoles ≥ 5/20	2	1	
unifocal ventricular extrasystoles ≥ 5/20	5	2	
multifocal ventricular extrasystoles 2—4/20	1	0	
unifocal ventricular extrasystoles 2—4/20	4	2	
occasional ventricular extrasystoles	8	13	
supraventricular extrasystoles ≥ 5/20	1	0	
supraventricular extrasystoles 2—4/20	1	1	
occasional supraventricular extrasystoles	0	7	
both supraventricular and ventricular extrasystoles	4	2	
	89	120	2

Table 18. Arrhythmias on admission to hospital (extrasystoles excluded)

	Males	%	Females		Total	
none	64	59,3	82	61 7	146	60
combinations	0		0		0	
ventricular tachycardia	5	4 6	0		5	2
atrial fibrillation	15	13 9	15	11,2	30	12
supraventricular tachycardia	1		0		1	0
idioventricular rhythm	1		2		3	1,
A—V nodal rhytm	0		2		2	[illegible]
sinus tachycardia	8		14		22	[illegible]
sinus bradycardia	2		3		5	2
other arrhytmias	0		2		2	0,

Table 19 A—V-conduction defects on admission to hospital

	Males	Females	Total	%
no	85	114	199	82,6
III	2	1	3	1,2
II	1	1	2	0,8
I P—R more than 0,22 s.	1	4	5	2,1
short P—R less than 0 12 s.	0	1	1	0 4
	89	120	209	

Table 20 Ventricular conduction defects on admission to hospital

	Males	Females	Total	
LBBB	5	9	14	5,8
RBBB	6	4	10	4 1
LAHB	4	4	8	3,3
LPHB	0	4	4	1 7
RBBB+ LAHB or LPHB	1	2	3	1,2
partial RBBB	0	3	3	1,2

had ventricular extrasystoles on admissio Supraventricular extrasystoles were discovere in 6 men (5 6 %) and 10 women (7 5 %) Ta ble 17

Comments
Puska et al (1973) found the incidence o extrasystoles (1 10 or more) to be 7 3 % i men and 6,6 % in women

Other arrhythmias
An arrhythmia (extrasystoles excluded) wa shown on the ECG on admission in 25 me (23 1 %) and 38 women (28 6 %) The mo common was *atrial fibrillation* which wa present in 15 men and women making a tota of 30 patients (12,4 %) Table 18

A—V conduction disturbances were rare occurring in only 4 men and 7 women. Com plete heart block was present in 2 men and 1 woman (i e 3 patients or 1,2 %) 2 patients (0 8 %) had second degree block on admissio and 5 patients (2 1 %) had first degree block (Table 19)

Ventricular conduction disturbances The most common was LBBB which was present in

-cases and conversion to sinus rhytm was achieved on 7 occasions.

Comments

The incidence of ventricular fibrillation in Lawries' series (1968) was 3 3 %. Dhurander (1971) has investigated ventricular fibrillation in Toronto, 20 patients out of total of 851 cases of infarction developed primary ventricular fibrillation. 11 of these were elderly patients. There were 9 patients in the 61—70 year age group and 5 of these survived. The only patient in the 71—80 year age group survived and the only patient (a male) in the 81—90 year age group died. These results are very similar to the results obtained in the present series.

Dhurander (1971) found that more than half of the cases of ventricular fibrillation started with multiple ventricular extrasystoles, but that 25 / may occur without any warning arrhytmia. Ventricular fibrillation after ventricular tachycardia occurred after ventricular tachycardia in 20 / of cases.

Raftery et al. (1972) noticed 23 cases of ventricular fibrillation, of whom 0 (45 %) died, in their series of 252 patients in coronary care unit. Ventricular fibrillation occurred after ventricular extrasystoles in 6 patients and 3 of these died (50 %) Most cases of ventricular fibrillation occurred after inferior infarction

Summary

Ventricular fibrillation is a serious complication. Primary treatment was succesfull in 8 patients (50 %)

Ventricula tachycardia

Ventricular tachycardia was present in 18 patients (7 5 /) and 1 of these died (6 1 %) in hospital Table 24 Ventricular tachycardia often preceded ventricular fibrillation.

Comments

Raftery et al. (1969) found 15 cases of uncomplicated ventricular tachycardia of whom 5 patients died in their series of 252 patient. Ventricular tachycardia occurring with ventricular extrasystolia was present in 7 patients in their series and 4 of these died (59 /)

Beck et al. (1975) noticed 2 cases of ventricular tachycardia one of whom died, in their series of 71 patients aged over 80 years.

Summary

The present study comprised all cases of ventricular tachycardia including those associated with other complications (often ventricular fibrillation) There was a high mortality associated with ventricular tachycardia or its associated complications.

Asystole

Table 24 shows that 3 patients had asystole One woman devoloped asystole followed by ventricular fibrillation during radiological examination. The patient was resuscitated and lived for a further three years. All the other patients died

Comments

Beck et al s series (1975) contained 4 cases of asystole out of 7 patients over 80 years old. All 4 patients died

Summary

Asystol was a serious complication and only one patient with this finding survived.

Shock

Shock was present in 7 men (6 5 %) and 17 women (2,8 /) and all died. Table 24.

Comments

Johansson (1972) found that shock was present in 205 men (9 5) and 119 women (1 /) in Malmö Beck et al. (1975) noticed

Table 24. Complications occurring during the period of hospitalization and their relationship to hospital mortality

	Males	%	died	%	Females	%	died	%	Total	%
heart failure	11	10,1	4	36,4	7	5,3	6	85,7	18	7,5
pulmonary oedema	12	11 1	6	50,0	17	12,8	12	70,6	29	12,0
shock	7	6,5	7	100,0	17	12,8	17	100,0	24	10,0
asystol	12	11 1	12	100,0	19	14,3	18	94,7	31	12,9
ventricular tachycardia	11	10,1	6	54,5	7	5,3	5	71 4	18	7,5
ventricular fibrillation	9	8,3	6	65,0	7	5,3	5	71 4	16	6,0

14 TEMPERATURE

Temperature rose 2—4 days after the infarc tion and the average maximum temperature in men was 38,1± 0 6 C and in women 37 9± 0 6 C.

15 LOCALIZATION OF INFARCTION AND ITS RELATIONSHIP TO HOSPITAL MORTALITY

Table 22 shows that 139 patients (i e. more than half) had anterior wall infarctions and 47 patients had inferior wall infarctions. Ex amination of the mortality associated with dif ferent localizations of infarction revealed that in women the mortality from anterior wall infarction was significantly less than from inferior wall infarction ($p<0{,}01$) There was no statistically significant difference in men (N S.D)

The extent of infarction and its relationship to hospital mortality

The majority of infarctions were transmural occurring in 160 cases (66 %) There were 48 cases (20 %) of subendocardial infarction Table 23 There was a statistically significantly greater mortality from transmural infarction than from subendocardial infarction in ($p<0{,}01$) but not in men (N.S.D) W had a significantly lower incidence of tran mural infarction than men ($p<0{,}01$) but suben docardial infarction was more common women.

Comments

Beck's series (1975) contained 56 cases transmural infarction of whom 40 died. Th were 11 cases of infarction other than trans mural infarction and 2 cases of these died.

There were 31 posterior wall infarctions, 21 of whom died and 29 anterior wall infarc tions of whom 19 died. The series also con tain~d 10 cases of recurrent infarctions of whom 5 died.

16 COMPLICATIONS OCCURRING DURING THE PERIOD OF HOSPITALIZATION

Ventricular fibrillation 16 patients (6 7 %) had ventricular fibrillation. Table 24. Primary treat ment was successfull in 8 patients (50 %) and a long lasting result was achieved in 5 patients (31,2 %) Ventricular fibrillation reverted to s nus rhytm in one case following a thump on the chest. Defibrillation was attempted in 13

Table 22. Localization of infarction (ECG) and its relationship to hospital mortality

	Males	%	died	%	Females	%	died	Total
anterior infarction	20		6		25		12	45
anteroseptal	16		8		26		6	42
anterolateral	21		5		31		8	52
lateral	2		1		3		1	5
inferior	23		10		23		13	46
inferolateral	6		1		8		4	14
posterior	0		0		1		0	1
other	1		0		1		0	2
unknown	19				15			34
	108				133			241

Table 23 The extent of infarction and its relationship to hospital mortality

	Males		died		Females		died		Total
transmural infarction	82	75,9	29	35 4	78	58,6	34	43,6	160
subendocardial infarction	11	10,2	2	18,2	37	27,8	8	21 6	48
unknown	15				18				33
Total	108				133				241

cases and conversion to sinus rhytm was achieved on 7 occasions

Comments
The incidence of ventricular fibrillation in Lawries' series (1968) was 3,3 %. Dhurander (1971) has investigated ventricular fibrillation in Toronto. 20 patients out of total of 851 cases of infarction developed primary ventricular fibrillation 11 of these were elderly patients There were 9 patients in the 61—70 year age group and 5 of these survived. The only patient in the 71—80 year age group survived and the only patient (a male) in the 81—90 year age group died. These results are very similar to the results obtained in the present series.

Dhurander (1971) found that more than half of the cases of ventricular fibrillation started with multiple ventricular extrasystoles but that 25 % may occur without any warning arrhytmia. Ventricular fibrillation after ventricular tachycardia occurred after ventricular tachycardia in 20 % of cases.

Raftery et al. (1972) noticed 23 cases of ventricular fibrillation, of whom 10 (45 %) died, in their series of 252 patients in a coronary care unit Ventricular fibrillation occurred after ventricular extrasystoles in 6 patients and 5 of these died (50 %) Most cases of ventricular fibrillation occurred after inferior infarction

Summary
Ventricular fibrillation is a serious complication. Primary treatment was successfull in 8 patients (5 %)

Ventricular tachycardia
Ventricular tachycardia was present in 18 patients (7 5 %) and 11 of these died (6 1 %) in hospital Table 24. Ventricular tachycardia often preceded ventricular fibrillation.

Comments
Raftery et al. (1969) found 15 cases of uncomplicated ventricular tachycardia of whom 5 patients died in their series of 252 patient. Ventricular tachycardia occurring with ventricular extrasystolia was present in 7 patients in their series and 4 of these died (59 %)

Beck et al. (1975) noticed 2 cases of ventricular tachycardia, one of whom died in their series of 71 patients aged over 80 years.

Summary
The present study comprised all cases of ventricular tachycardia including those associated with other complications (often ventricular fibrillation) There was a high mortality associated with ventricular tachycardia or its associated complications.

Asystole
Table 24 shows that 31 patients had asystole. One woman devoloped asystole followed by ventricular fibrillation during radiological examination. The patient was resusciated and lived for a further three years. All the other patients died.

Comments
Beck et al.s series (1975) contained 4 cases of asystole out of 71 patients over 80 years old. All 4 patients died.

Summary
Asystole was a serious complication and only one patient with this finding survived.

Shock
Shock was present in 7 men (6 5 %) and 17 women (12,8 %) and all died. Table 24.

Comments
Johansson (1972) found that shock was present in 105 men (9 5 %) and 119 women (11 %) in Malmo. Beck et al. (1975) noticed

Table 24 Complications occurring during the period of hospitalization and their relationship to hospital mortality

	Males	%	died	%	Females	%	died	%	Total	%
heart failure	11	10 1	4	36 4	7	5,3	6	85 7	18	7,5
pulmonary oedema	12	11 1	6	50,0	17	12,8	12	70,6	29	12,0
shock	7	6,5	7	100,0	17	12,8	1	100,0	24	10,0
asystole	12	11 1	12	100,0	19	14,3	18	94,7	31	12,9
ventricular tachycardia	11	10 1	6	54,5	7	5,3	5	71 4	18	7,5
ventricular fibrillation	9	8,3	6	65,0	7	5,3	5	71 4	16	6,0

16 cases of cardiogenic shock in their series [71 patients over 80 years old 15 of these died.

Summary
Cardiogenic shock *occurring after infarction is* a complication which almost always leads to death in elderly patients

Heart failure
Heart failure occurred after myocardial in*farction in 18* patients (7 5 %) 10 of these died (55 6 %) Table 24.

Pulmonary oedema was present in 39 patients (16 2 %) and 18 of these died (46 1 %) Table 24

Comments
The development of heart failure following infarction was a sign of a poor prognosis. Beck et al (1975) in their series of 71 patients over 80 years old found 66 cases *of heart failure and 42 of these died. 18 had* pulmonary oedema and 13 of these died. The results are similar to those in the present series.

Summary
Pulmonary oedema was common and primary treatment *was succesfull in 46 1 % of cases.*

Ventricular extrasystoles
Frequent ventricular extrasystoles (more than 5 per minute *R on T or consecutive* ventricular extrasystoles) were present in 38 patients (15 8 %) and 12 (31 6 %) of these died. Table 25

Comments
Raftery et al s series (1969) of 252 patients in a coronary care unit contained 70 patients with ventricular extrasystoles and 15 (2) of these died. *Most* cases of ventricular extrasystoles (42 patients) followed anterior wall infarction

Beck et al (1975) found 19 cases of ventricular extrasystoles of which 15 died in the series of 71 patients over 80 years old.

Summary
In the present study only frequent occurring *extrasystoles* or those requiring treatment were included and their association to hospital mortality was high.

Atrial fibrillation
Atrial fibrillation occurred in 20 patients (8,3 %) and 8 of these died (40 %) Atrial fibrillation resolved spontaneously in 10 patients. One patient benefitted from digitalisation. DC conversion succeeded in restoring sinus rhythm in another patient. *Table 25*

Comments
Atrial fibrillation was present in 21 patients in Beck et al s study (1975) and 16 of these died in hospital Atrial fibrillation worsens the prognosis.

Helmers (1973) investigated the incidence of atrial fibrillation occurring during the first *24 hours in a coronary care unit. 15 % of the* 450 patients with infarction in his series had atrial fibrillation and there was an associated hospital mortality rate of 38 % while the mortality for other patients with infarction was 16 %. Recurrent episodes did not increase the mortality Patients with atrial fibrillation tended to be older and often had left ventricular failure. The highest incidence of atrial fibrillation was in *the older* age groups.

Klass (1970) found a 7 5 % incidence of atrial fibrillation *and the mortality in his series* among these patients was 42 %

Summary
The results in the present study in relation to the incidence and mortality of atrial fibrillation are very similar to those obtained in *other studies.*

Table 25. *Various arrhythias during the period of hospitalization and their relationship to hospital mortality*

	Males		died		Females		died	Total	
ventricular extrasystoles (frequent, coupled R-on T-wave)	20	18,5	4	20,0	18	19,		38	15
atrial fibrillation	11	10,2	5	45,5	9				
total A – V-block	4		1	25 0	1				
I II -block	1		0		3				
supraventricular tachycardia	1		1		2				
sinusbradycardia	2		0		3				

Other complications

Table 25 reveals that 5 patients (2 %) had complete A—V-block. One of these patients improved when digitalis was discontinued, one responded to isoprenaline and 2 patients required a pacemaker. One of these soon died of severe heart failure, but the other was still alive three years. The postinfarction syndrome was diagnosed in one patient only. Two patients had parasystolia.

Comments

.. (1969) has studied the incidence of A—V-block occurring after both anterior .. posterior wall infarction. The investigation was carried out in a coronary care unit and the patients' ages ranged from 40—69 years in those with posterior wall infarction and 47—66 years in those with anterior wall infarction. The infarction was transmural in all patients. A—V-block resolved spontaneously in 1—3 days in uncomplicated cases more frequently in posterior wall infarction. Adams-Stokes attacks were uncommon in these patients and the QRS-complex was of normal duration in third degree A—V-block.

In anterior wall infarction A—V-block is usually complete, Adams-Stokes attacks are common and the QRS-duration is prolonged. First degree block often precedes or follows other types of block while complete A—V block in anterior wall infarction often occurs after right bundle branch block. Pacing is not necessary for heart block occurring in posterior wall infarction.

17 RADIOLOGICAL CHANGES IN PATIENTS WITH MYOCARDIAL INFARCTION

Because radiological assistance was difficult to obtain, 131 consecutive myocardial infarction cases were studied in detail. Chest roentgenograms were obtained from .. of these patients (52 men and 59 women). Roentgenograms were obtained from 85,4 % of the patients.

A chest roentgenogram was taken on average 4,4± 4,1 days after infarction in men and 4,2± 4,2 days after infarction in women.

Heart size is difficult to assess when the patient is recumbent, and comparison between erect and recumbent films also presents difficulties. An attempt was made to overcome this problem by using different constants for roentgenograms taken in the erect and recumbent position. The results obtained, however, must be interpreted with caution.

The mean total heart volume in men was 1164± 343 ccm and in women 875± 229 ccm. The mean volume in men was significantly greater than in women ($p<0{,}001$). The heart volume compared to the surface area of the skin was 632± 211 ccm/m² in men and 537± 123 ccm/m² in women ($p<0{,}001$).

Enlargement of different chambers of the heart heart

Heart size was normal in 11 men and 14 women in hospital. Table 26. There was no difference between the sexes in enlargement of different part of the heart (NSD). Heart enlargement was observed in about 80 % of patients.

Radiological evaluation of pulmonary venous congestion

Table 27 shows that venous congestion is was normal in 25 men (27,8 %) and 34 women (46,6 %). The pulmonary vasculature was normal almost significantly more often in women than in men ($p<0{,}05$).

Table 27 shows that venous congestion is more common in men as measured by various parameters.

Classification of pulmonary venous congestion was performed according to Turner's method (Turner et al. 1972). There was no statistical

Table 26. Enlargement of the different chambers of the heart

	Males	%	Females		Total
No enlargement	11	18,0	14	20,6	25
left auricle	3	4,9	9	13,2	12
left ventricle	23	37,7	24	35,3	47
right ventricle	5	8,2	6	8,8	11
combinations common enlargement	19	31,1	15	22,1	34

16 cases of cardiogenic shock in their series (71 patients over 80 years old 15 of these died.

Summary
Cardiogenic shock occurring after infarction is a complication which almost always leads to death in elderly patients

Heart failure
Heart failure occurred after myocardial in farction in 18 patients (7 5 %) 10 of these died (55,6 %) Table 24

Pulmonary oedema was present in 39 pa tients (16,2 %) and 18 of these died (46 1 %) Table 24

Comments
The development of heart failure following infarction was a sign of a poor prognosis. Beck et al. (1975) in their series of 71 patients over 80 years old found 66 cases *of heart failure and 42 of these died. 18 had* pulmonary oedema and 13 of these died. The results are similar to those in the present series.

Summary
Pulmonary oedema was common and primary treatment was succesfull in 46 1 % of cases.

Ventricular extrasystoles
Frequent ventricular extrasystoles (more than 5 per minute R on T or consecutive ventri *cular extrasystoles) were present in 38 patients* (15,8 %) and 12 (31,6 %) of these died. Table 25

Comments
Raftery et al s series (1969) of 252 patients in a coronary care unit contained 70 patients with *ventricular extrasystoles and 15 (2) of* these died. Most cases of ventricular extra systoles (42 patients) *followed anterior wall* infarction.

Beck et al. (1975) *found 19 cases of venu cular extrasystoles of which 15 died in* series of 71 patients over 80 *years old*

Summary
In the present study only frequent occurring extrasystoles *or those requiring treatment,* were included and their association to hospital mortality was high

Atrial fibrillation
Atrial fibrillation occurred in 20 patients (8 3 %) and 8 of these died (40 %) Atrial fibrilla tion resolved spontaneously in 10 patients. One patient benefitted from digitalisation D C *conversion succeeded in restoring sinus rhytm* in another patient. Table 25

Comments
Atrial fibrillation was present in 21 patients *in Beck et al. s study (1975) and 16 of these* died in hospital Atrial fibrillation worsens the prognosis.

Helmers (1973) investigated the incidence *of atrial fibrillation occurring during the first 24 hours in a coronary care unit. 15 % of the* 450 patients with infarction in his series had atrial fibrillation and there was an associated *hospital mortality rate of 38 %* while the mor tality for other patients with infarction was 18 % Recurrent episodes did not increase the mortality Patients with atrial fibrillation tend *ed to be older and often had left ventricular* failure. The highest incidence of atrial fibrilla tion was in the older age groups.

Klass (1970) *found a 7 5 % incidence of at rial fibrillation* and the mortality in his series among these patients was 42 %.

Summary
The results in the present study in relation to the incidence and mortality of atrial fibril lation are very similar to those obtained in *other studies.*

Table 25. Various arrhythias during the period of hospitalization and their relationship t hospital m rtality

	Males		died		Females		died		T tal	
ventricular extrasystoles (frequent, coupled, R-on T-wave)	20	18,5	4	20 0	18	13,5	8	44 4	38	15,8
atrial fibrillation	11	10,2	5	45,5	0	6,7	3	33,3	20	8,3
total A—V-block	4		1	25 0	1		0		5	2 1
I II -block	1		0		3		0		4	1 7
supraventricular tachycardia	1		1		2		0		3	1,2
sinusbradycardia	2		0		3		0		5	2 1

ally significant difference between men and ~n Table 28 The post-capillary component was normal in 18 men (34,6 %) and 25 women (42,4 %) Pulmonary oedema was present in 16 men (30 %) and 8 women (13,6 %)

The pre-capillary component was normal in 29 men (55,8 %) and 34 women (45,8 %) N S D) Table 29

The major branches of the pulmonary arteries were normal in 14 men (26,9 %) and 27 women (45,8 %) Table 30.

The mean pulmonary arterial pressure obtained using Turner's method was 31,9± 22 5 mmHg in men and 47 3± 20 7 mmHg in women (N.S.D)

Heart calcifications
Coronary calcifications were fairly common occurring in 7 men (13 5 %) and in 19 women (32,2 %) and the difference between men and women was almost significant ($p < 0{,}05$) Table 31 Myocardial calcification was observed in at least one man.

Valvular calcifications was also rare. One woman had mitral valvular calcification and two women had aortic valvular calcification, but no valvular calcification was observed in men

Aortic calcification was quite common especially in women Table 32

7 men (3 5 %) and 27 women (45,8 %) had atherosclerotic calcification. The difference is statistically significant ($p < 0{,}00$) In addition, two men had syphilitic aortic calcification. Aortic aneyrysm was not observed

Left ventricular aneyrysm was suspected in the chest roentgenogram in one man and two women

Comments
Ruikka et al (966) found aortic calcification to be present in o % of men and 24 % of women

18 POST MORTEM FINDINGS

Number of cases
Of the patients examined clinically 40 men (3 ,0 %) died in hospital and post-mortem examination was performed on 33 of these (82 5 %) 52 women (39 1 %) died in hospital and post-mortems were performed on 38 of these patients (71 7 %) Altogether 71 patients (77,4 %) underwent post-mortem examination, which represented a fairly high level internationally

Heart weight averaged 594,4± 135 1 g in men and 454,1± 103,0 g in women. In men the heart weighed significantly more than in women ($p < 0{,}001$)

Pathological diagnosis
Recent myocardial infarction was found in 30 men (90,9 %) and 36 women (94,7) Table 33

Old infarction or infarct scars were significantly more common in men, being present in men (75,8 %) and 17 women (44 7 %) ($p < 0{,}001$)

Age and location of infarction
Tables 34 and 35 show that the distribution of infarctions in the different areas is fairly even Only 3 men and one woman had recent myocardial infarction in the right ventricle. Recent infarct was present in 44 1 of the men and 59,6 % of the women. Granulations were present in men 15 9 % and in women 9 9

Old infarct scars were present in 42 patients altogether (59 1 %)

Coronary artery findings
There was moderate sclerosis in the right coronary artery in 1 men (65,6 %) and 16 women (42 1 %) Recent thrombus of the right coronary artery was present in 8 men (24,2 %) and 11 women (28,9 %)

Recent thrombus in the region of the main branch of the left coronary artery was found in only one man and two women. Thrombus was present in the region of the descending

Table 32 Radiological aortic calcification

	Males		Females	%	Total	%
no calcification	43	82,7	32	54,2	75	67 6
atherosclerotic	7	13,5	27	45,8	34	30,6
syphilitic	2	3,8	0		2	1,8
aortic aneurysm	0		0		0	

Table 27 Roentgenological evaluation of pulmonary venous congestion

	Males		Females		Tot
none	25	27,8	34	46,0	5(
prominent vasculature	4	4 4	9	12,3	13
interstitial oedema	9	10,0	8	11,0	17
alveolar oedema (butterfly)	17	18 9	8	11,0	25
interlobar oedema	13	14 4	7	9 6	20
hydrothorax·	8	8,9	3	4 1	11
left	8	8 9	2	2,7	10
right	6	6 7	2	2,7	8

Table 28. Post-capillary component according to Turner

	Males		Females		Total
normal 5—10 mmHg	18	34 6	25	42,4	43
1+ Redistribution equal perfusion of the upper and lower lung zones = 10—15 mmHg	7	13,5	11	18 6	18
2+ Redistribution greater perfusion in the upper than the lower lung zones = 15—25 mmHg	6	11,5	7	11,9	13
3+ Redistribution. interstitial oedema = 25—35 mmHg	5	9,5	8	13 6	13
4+ Redistribution alveolar oedema = over 35 mmHg	16	30,8	8	13,6	24

Table 29 Pre-capillary component according to Turner
The main pulmonary artery·

	Males	%	Females		Total
normal, 15 mmHg	29	55,8	34	58,6	63
1+ enlarged = 15+10 = 25 mmHg	11	21,2	17	29,3	28
2+ enlarged = 15+20 = 35 mmHg	9		7		16
3+ enlarged = 15+30 = 45 mmHg	3		0		3

Table 30 The major branches of the pulmonary arteries

	Males		Females	%	Total
normal	14	26,9	27	45,8	41
proximal dilatation = 5 mmHg	21	40 4	22	37,3	43
distal attenuation = 15 mmHg	17	32,7	10	16 9	27
	52		59		111

Table 31 Radiological heart calcifications

	Males	%	Females	%	Total	
no calcifications	44	84,6	40	67,8	84	75 7
coronary calcifications	7	13,5	19	32,2	26	23 4
myocardial calcifications	1	1 8	0		1	
pericardial calcifications	0		0		0	

cally significant difference between men and women. Table 28. *The post-capillary component* was normal in 18 men (34,6 %) and 25 women (42,4 %). Pulmonary oedema was present in 16 men (30 %) and 8 women (13,6 %).

The pre-capillary component was normal in 29 men (55,8 %) and 34 women (45,8 %) (N.S.D.) Table 29.

The major branches of the pulmonary arteries were normal in 14 men (26,9 %) and 27 women (45,8 %). Table 30.

The *mean pulmonary arterial pressure* obtained using Turner's method was 51,9 ± 22,5 mmHg in men and 47,3 ± 20,7 mmHg in women (N.S.D.).

Heart calcifications
Coronary *calcifications* were fairly common, occurring in 7 men (13,5 %) and in 19 women (32,2 %) and the difference between men and women was almost significant (p<0,05). Table 31. Myocardial calcification was observed in at least one man.

Valvular calcifications was also rare. One woman had mitral valvular calcification and two women had aortic valvular calcification, but no valvular calcification was observed in men.

Aortic calcification was quite common especially in women. Table 32.
7 men (13,5 %) and 27 women (45,8 %) had atherosclerotic calcification. The difference is statistically significant (p<0,001). In addition, two men had syphilitic aortic calcification. Aortic aneurysm was not observed.

Left ventricular aneurysm was suspected in the chest roentgenogram in one man and two women.

Comment
Ruikka et al. (1966) found aortic calcification to be present in 10 % of men and 24 % of women.

Table 32. Radiological aortic calcification

	Males	%	Females	%	Total	%
no calcification	43	82,7	32	54,2	75	67,6
atherosclerotic	7	13,5	27	45,8	34	30,6
syphilitic	2	3,8	0		2	1,8
aortic aneurysm	0		0		0	

18 POST MORTEM FINDINGS

Number of cases
Of the patients examined clinically 40 men (37,0 %) died in hospital and post-mortem examination was performed on 33 of these (82,5 %). 52 women (39,1 %) died in hospital and post-mortems were performed on 38 of these patients (71,7 %). Altogether 71 patients (77,4 %) underwent post-mortem examination which represented a fairly high level internationally.

Heart weight averaged 594,4 ± 135,1 g in men and 454,1 ± 103,0 g in women. In men the heart weighed significantly more than in women (p<0,001).

Pathological diagnosis
Recent myocardial infarction was found in 30 men (90,9 %) and 36 women (94,7). Table 33.

Old infarction or infarct scars were significantly more common in men being present in men (75,8 %) and 17 women (44,7 %) (p<0,001).

Age and location of infarction
Tables 34 and 35 show that the distribution of infarctions in the different areas is fairly even. Only 3 men and one woman had recent myocardial infarction in the right ventricle. Recent infarct was present in 44,1 % of the men and 59,6 % of the women. Granulations were present in men 15,9 % and in women 9,9 %.

Old infarct scars were present in 42 patients altogether (59,1 %).

Coronary artery findings
There was moderate sclerosis in the right coronary artery in 21 men (63,6 %) and 16 women (42 %). Recent thrombus of the right coronary artery was present in 8 men (24,2 %) and 11 women (28,9 %).

Recent thrombus in the region of the main branch of the left coronary artery was found in only one man and two women. Thrombus was present in the region of the descending

Table 33. Pathological findings in the heart at post mortem examination

	Males	%	Females	%	Total	
recent infarction	30	90,9	36	94 7	66	93,
recent thrombus	21	63 6	20	52,6	41	57,
old coronary thrombosis	6	18,2	3	7 9	9	12,
old infarction or -scar	25	75,8	17	44 7	42	59
severe stenosis (over 50)	23	69 7	22	57 9	45	63,4

Table 34 Age and location of infarction in men

	septal		anterior		lateral		posterior		right ventricle	Total	
	apical	basal	apical	basal	apical	basal	apical	basal			
recent infarction	11	7	16	10	9	6	12	12	3	86	44,1
granulation	6	4	5	2	4	1	6	3	0	31	15,9
scar	8	10	10	10	9	10	11	10	0	78	40,0
Total	25	21	31	22	22	17	29	25	3	195	

Table 35 Age and location of infar-tion in women

	septal		anterior		lateral		posterior		right ventricle	Total	
	apical	basal	apical	basal	apical	basal	apical	basal			
recent infarction	15	10	12	12	15	13	16	14	1	108	59,6
granulation	3	1	3	0	3	1	4	3	0	18	9,9
scar	8	8	8	3	5	3	12	8	0	55	30,4
Total	26	19	23	15	23	17	32	25	1	181	

Table 36. Coronary artery findings in men

	right coronary artery	the main branch of left coronary artery	the descending branch of left coronary artery	the left circum flex branch	Total
moderate sclerosis	21	22	16	10	78
stenosis (over 90 %)	5	3	7	5	20
old thrombus	2	0	2	1	5
recent thrombus	8	1	9	1	19
	36	26	34	26	122

Table 37 Coronary artery findings in women

	right coronary artery	the main branch of left coronary artery	the descending branch of left coronary artery	the left circum-flex branch	T tal
moderate sclerosis	16	20	15	19	70
stenosis (over 90)	6	5	12	9	32
old thrombus	2	0	1	0	3
recent thrombus	11	2	7	2	22
	35	27	35	30	27

Table 28. Other findings at post-mortem examination

	Males	Females	Total	%
ventricular aneurysm	5	2	7	9,9
ventricular rupture	5	3	8	11,3
septum perforation	0	0	0	
papillary muscle rupture	0	0	0	
mural thrombus	6	5	11	15,5
cerebral embolus	2	0	2	2,8
coronary embolus	0	1	1	1 4
limb embolus alone	0	0	0	
mural thrombus + limb- or other embolus	2	2	4	5,6
other embolus	1	8	9	12,7

branch in 9 men and 7 women and in the region of the circumflex branch in one man and two women Tables 36 and 37

Moderate coronary sclerosis was observed in 63,9 / of the men and in 55,1 / of the women. Over 90 / occlusion of the coronary artery was present in 16,4 / of the men and in 25,2 /o of the women. Old thrombus was observed in 4 / of the men and in 2,4 / of the women. Recent thrombus occurred in 15,6 / of the men and in 17 3 / of the women.

Oth r findings discovered at post mortem examination

Ventricular aneurysm was discovered in 5 men and 2 women of those patients examined clinically The difference is not tatistically significant. Ventricular rupture was present in 5 men and 3 women giving total of 8 patients (11 /o) (N S D.) Mural thrombus was found in 6 men and 5 women, making 1 patients altogether (14,6 /o) Two men had cerebral embolism Coronary embolus was discovered in one woman Other emboli were present in one man and 8 women (N S D.) Table 38

Comments

Montonen (969) found old infarct scars in 55 of men and n 49 3 % of women. In his series coronary sclerosis was discovered most frequently in the region of the left descending branch in patients aged 70—74 years (47 7) and in the region of the right coronary artery t the age of 75—79 years (47 7 /) Sclerosis was most common in th region of the left circumflex branch at the age of 75—79 years, occurring in 53,2 /

He found entricular rupture in 52 patients (2 96 %) Ventricular rupture was present almost significantly more commonly in the region f the anterior wall than in the posterior wall There were significantly more old infarct scars in the region of the posterior wall.

Taschel (1972) observed that after 70—80 years of age, ventricular rupture occurred principally in old women. He noticed a conflict between the initial clinical symptoms and the histological picture. He surmised that the infarction is not noticed at the beginning and the symptoms attributed to the infarction are actually due to the rupture.

Oblath et al. (1952) found ventricular rupture in 58,8 / of women and in 41,2 % of men. All patients were over 50 years old. Sievers (1963) found that ventricular rupture increased with increasing age and that rupture occurred in women more frequently than in men. In a large scale postmortem series ventricular rupture was observed in about 1 % of cases (Oblath et al., 1952 0 53 % London et al., 1965 1,4 %)

In Bjorck's (1972) series of 529 consecutive cases of myocardial infarction 19 cases of ventricular rupture were discovered. No patient had had previous infarction. There were no cases of inferior infarction. Seven of the 11 cases of ventricular rupture occurred during the first day None of the cases of ventricular rupture followed massive infarction which would suggest that these cases may be amenable to surgical treatment.

Summary

Post mortem examination showed that myocardial infarction in both men and women was present equally frequently in the anteroseptal and posterolateral wall of heart.

Sclerosis was equally common in the regions of th different coronary arteries in both men and women. Recent thrombus was discovered most frequently in the region of th right coronary artery and the left descending branch

Table 33. Pathological findings in the heart at post-mortem examination

	Males	%	Females		Total	%
recent infarction	30	90 9	36	94 7	66	93,0
recent thrombus	21	63 6	20	52,6	41	57 7
old coronary thrombosis	6	18,2	3	7 9	9	12 7
old infarction or -scar	25	75,8	17	44 7	42	59,2
severe stenosis (over 50 %)	23	69 7	22	57,9	45	63 4

Table 34 Age and location of infarction in men

	septal		anterior		lateral		posterior		right ventricle	Total	
	apical	basal	apical	basal	apical	basal	apical	basal			
recent infarction	11	7	16	10	9	6	12	12	3	86	44,0
granulation	6	4	5	2	4	1	6	3	0	31	15,0
scar	8	10	10	10	9	10	11	10	0	78	40,0
Total	25	21	31	22	22	17	29	25	3	195	

Table 35 Age and location of infar tion in women

	septal		anterior		lateral		posterior		right ventricle	Total	
	apical	basal	apical	basal	apical	basal	apical	basal			
recent infarction	15	10	12	12	15	13	16	14	1	108	59,6
granulation	3	1	3	0	3	1	4	3	0	18	9 9
scar	8	8	8	3	5	3	12	8	0	55	30 4
Total	26	10	23	15	23	17	32	25	1	181	

Table 36 Coronary artery findings in men

	right coronary artery	the main branch of left coronary artery	the descending branch of left coronary artery	the left circum flex branch	Total
moderate sclerosis	21	22	16	19	78
stenosis (over 90 %)	5	3	7	5	20
old thrombus	2	0	2	1	5
recent thrombus	8	1	9	1	19
	36	26	34	26	122

Table 37 Coronary artery findings in women

	right coronary artery	the main branch of left coronary artery	the descending branch of left coronary artery	the left circum-flex branch	T tal
moderate sclerosis	16	20	15	19	79
stenosis (over 90 %)	6	5	12	9	32
old thrombus	2	0	1	0	3
recent thrombus	11	2	7	2	22
	35	27	35	30	127

Table 40. Sauna

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
Does not go to sauna	32	49	81	8	17	25
Once a month	7	12	19	3	8	11
Every other week	14	12	26	7	13	20
Once a week	30	33	63	15	16	31
2–3 times a week	11	0	11	3	3	6
4–5 times a week	1	0	1	0	0	0
Unknown	13	27	40	2	5	7
	108	133	241	38	62	100

Sauna

Table 40 reveals that the majority of patients frequented the sauna. There was a significant difference between the female patients and female controls ($p<0{,}05$) i.e. the former group contained more patients who did not go to the sauna at all.

Frequenting the sauna does not appear at any rate to increase the amount of myocardial infarctions.

Sugar consumption

The fact that there were significantly more diabetics in the patient group explains why this group contained more patients who did not take sugar (27 men and 8 women in the patient group and one man and 7 women in the control group $p<0{,}001$)

Fat consumption

No difference was seen between patients and controls in the consumption of fats. There was almost significantly greater number of male patients with high fat consumption in comparison to female patients ($p<0{,}05$)

Consumption of beer, wine and spirits

No difference was observed between the control group and the patients in the consumption of beer, wine and spirits. However the men consumed significantly more of all kinds of alcohol than the women ($p<0{,}001$)

Comments

Studies on association between alcohol consumption and ischaemic heart disease have yielded conflicting results. Chronic alcoholics have been considered to possess protection from coronary atherosclerosis. Several authors found no association between alcohol consumption and ischaemic heart disease, also the Framingham Study (Kannel 1966)

In a mortality study on approximately one million persons under the age of 50 alcoholism and heavy drinking were overrepresented among fatal ischaemic heart disease cases (Bainton et al., 1963). A study from Norway has also shown a higher mortality from ischaemic heart disease among chronic alcoholics compared to the general population (Sundby 1967). In a report on men born in 1913 registration at the local temperance board showed a strong association with mortality in myocardial infarction (T bblin 1972)

21 ROLE OF THE RISK FACTORS

1 Hypertension

Hypertension is most important of the well known risk factors in myocardial infarction. Table 41 indicates that 11 men (10,2) and 3 women (24 1 /) had definite hypertension,

Table 41 Hypertension

	Patients with myocardial infarction			Controls		
	Males	Females	Total	Males	Females	Total
definit hypertension	11	32	43	4	10	14
not definite hypertension	0	8	8	1	3	4
no hypertension	83	84	167	33	46	79
unknown	14	9	23	0	3	3
	108	133	241	38	62	100

and rarely in the region of the main branch of left coronary artery and the left circumflex branch ($p<0,05$)

Analysis of different age groups were not performed because of the small size of these age groups. Sclerotic changes were present to the same extent as in Möttönen's series in the old age group Ventricular rupture was more common in this study than other series

19 DEMOGRAPHIC INFORMATION

Marital status

Many studies have shown that myocardial infarction is more common in unmarried people. In the present study there were no single men The control group contained two unmarried persons

Social class

No difference was noticed in male and female patients and the controls. Both patients and the controls came most commonly from the third social class However men outnumbered women in the first social class both in the patient group and in the control group The difference is not significant.

20 OTHER SOCIO ECONOMIC FACTORS

Place of birth

No significant difference was discovered in the development of myocardial infarction between the place of birth of the patients and of the controls It is interesting that Karelians in Turku including those who arrived 30 years ago (Finland was forced to cede part of Karelia to the Soviet Union after the Second World War) had an incidence of infarction similar to the other patients There were however so few true Karelians that it is difficult to draw definite conclusion.

Both patients and controls had lived in Tu ku city or some other town for about 50 ye on average (N.S.D)

Residential circumstances

Most of the patients 67 men (62 %) and 51 women (43 6 %) lived in their own res 16 men and 26 women had hire recidence In institutions lived 6 men and 25 women The residences were relatively comfortable more than half of both patients and contro had all modern conveniences (Only 5 and 15 women did not have modern conveniences) There was no statistically s difference between patients and controls

Patient's ability to look after himself before the infarction

The majority of both patients (86 men and 97 women) and controls (37 men and 50 women) were able to look after themselves 46 patients required help There was no statistically significant difference between men and women in this respect.

Pension

There were no differences between the patients and the controls receiving a pension. Women were economically worse off and received more supports and supplements.

Coffee consumption

The great majority of patients were coffee-drinkers. They numbered 168 as opposed to only 15 patients who did not as far as is known drink coffee. Similarly the control group contained only 5 persons who did not drink coffee. The male patients consumed a daily mean of $4,4 \pm 2\ 7$ cups while the mean consumption for female patients was $3\ 7 \pm 2\ 1$ cups. (Table 39) In the control group the men drank $4\ 6 \pm 2\ 8$ cups of coffee and the women drank $3,4 \pm 1\ 7$ cups. Consumption of coffee does not appear to be of significance in the development of myocardial infarction in this series.

Table 39 Coffee consumption

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
drink coffee	77	89	166	36	57	193
do not drink	8	7	15	2	3	5
unknown	23	37	60	0	2	2
	108	133	241	38	62	100

Table 40. Sauna

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
does not go to sauna	32	49	81	8	17	25
once a month	7	12	19	3	8	11
every other week	14	12	26	7	13	20
once a week	30	33	63	15	16	31
2—3 times a week	11	0	11	3	3	6
4—5 times a week	1	0	1	0	0	0
unknown	13	27	40	2	5	7
	108	133	241	38	62	100

Sauna
Table 40 reveals that the majority of patients frequented the sauna. There was a significant difference between the female patients and female controls ($p<0{,}05$) i.e. the former group contained more patients who did not go to the sauna at all.

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The fact that there were significantly more diabetics in the patient group explains why this group contained more patients who did not take sugar (7 men and 28 women in the patient group and one man and 7 women in the control group $p<0{,}001$)

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No difference was seen between patients and controls in the consumption of fats. There was almost significantly greater number of male patients with high fat consumption in comparison to female patients ($p<0{,}05$)

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No difference was observed between the control group and the patients in the consumption of beer wine and spirits. However the men consumed significantly more of all kinds of alcohol than the women ($p<0{,}001$)

Comments
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Hypertension is most important of the well known risk factors in myocardial infarction. Table 41 indicates that 11 men (10,2 %) and 3 women (24 t) had definite hypertension

Table 41 Hypertension

	Patients with myocardial infarction			Controls		
	Males	Females	Total	Males	Females	Total
definite hypertension	11	32	43	4	10	14
not definit hypertension	0	8	8	1	3	4
no hypertension	83	84	167	33	46	79
unknown	14	9	23	0	3	3
	108	133	41	38	62	100

and rarely in the region of the main branch of left coronary artery and the left circumflex branch ($p<0{,}05$)

Analysis of different age groups were not performed because of the small size of these age groups. Sclerotic changes were present to the same extent as in Möttönen's series in the old age group. Ventricular rupture was more common in this study than other series.

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Both patients and controls had lived in Tu ku city or some other town for about 50 y on average (N.S.D.)

Residential circumstances

Most of the patients 67 men (62 %) and women (43,6 %) lived in their own residence 16 men and 26 women had hire-recidence In institutions lived 6 men and 25 women The residences were relatively comfortable and more than half of both patients and controls had all modern conveniences (Only 5 men and 15 women did not have modern conveniences) There was no statistically significant difference between patients and controls

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Table 39. Coffee consumption

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
drink coffee	77	80	160	36	57	103
do not drink	8	7	15	2	3	5
unknown	23	37	60	0	2	2
	108	133	241	38	62	100

reaching 96 % of the normal level after 7 weeks.

Enger et al. (1970) found that the cholesterol level was 9 days after infarction about % below the level 3 months after infarction.

The investigation was hampered by the fact that the cholesterol level was estimated during the period of convalescence in hospital when the level was approaching its lowest. In view of the fact that the majority of the serum cholesterol levels were estimated about 8 days after infarction, Ritland's (1975) calculation for the correction of this level was also performed. Mean cholesterol levels of 8,6 mmol/l for men and 9,5 mmol/l for women were obtained, and these levels are significantly higher than in the control group ($p<0{,}001$)

Summary On the basis of present study the high serum cholesterol level appeares possibly to be risk factor in the elderly

Triglycerides were investigated together with the serum cholesterol about 8 days after infarction. There was no information about levels prior to infarction in any patient.

The mean level in men was 1,63± 0,8 mmol/l and in women 1,60± 0,63 mmol/l (N.S.D.) 14 men and 11 women had levels over 1,7 mmol/l

In the control group the mean levels were 1,50± 0,51 mmol/l and 1,62± 0,69 mmol/l for men and women respectively. There was no statistically significant difference between the patients and the controls.

Comments

According to Ritland (1975) the triglyceride level rises after infarction to achieve a maximum level of 116 % of the normal level after 2 weeks. This increase was not statistically significant however

Summary

No statistically significant difference was seen when compared to the control group. The triglyceride level is thus probably not a significant risk factor

4 Physical Inactivity

More than half of patients with myocardial infarction (55 men and 74 women, altogether 129 patients) had limited physical activity. Only 28 (28 %) of the controls had limited physical activity. Spare time activity was similar in both groups.

The fact that elderly patients have many illnesses which curtail the physical activity makes it difficult to draw conclusions about the significance of physical inactivity

5 Obesity

In this study the body mass index (i.e. Quitelet's index) was used, in which the patients weight is divided height squared (Keys et al. 1972)

The controls were measured and weighed at the time of their examination. The patients with infarction had these measurements taken in hospital. Some of the information was obtained from the patients themselves.

The Quitelet's index in male patients was 2,39± 0,44 and in male controls 2,38± 0,74 (N.S.D.) The index in female patients was 2,45± 0,38 and in female controls 2,54± 0,39 (N.S.D.)

Summary

In this study body mass or overweight was not found to be associated with myocardial infarction. Overweight would thus not seem to be a risk factor in the elderly

6 Smoking

There were 26 male (24,1 %) and 5 female smokers making total of 31 patients (12,6 %) who smoked Table 43. There were significantly more male smokers than female smokers ($p<0{,}00$) The control group showed the same tendency

However the patient group did not contain more smokers than the control group.

Table 43. Smoking

	Patients with myocardial infarction			Controls		
	Males	Females	Total	Males	Females	Total
smokers	[illegible]		31	6	2	8
has stopped smoking earlier		[illegible]	47	25	3	28
never smoked		[illegible]	123	7	56	63
unknown		[illegible]	[illegible]	0	1	1
				38	62	100

making a total of 43 patients (17,8 %) Hypertension was present significantly more frequently in women than in men ($p<0{,}01$) Compared to the control group there was no significant difference between either the men or the women.

Comments

Vartio (1960) found a 15 1 % incidence of hypertension in their series. Puska et al (1974) reported a 11 8 % incidence of hypertension in men and 18,8 % incidence in women in North Karelia

Summary

The incidence of hypertension in patients over 65 years old was quite similar to that in North Karelia. Hypertension is an important risk factor in younger patients but in the present study it did not appear to be of significance in the elderly

2 Diabetes

Diabetes was confirmed in 20 men (18 5 %) and 33 women (24 8 %) There was a significantly higher incidence of diabetes both in the men and in the women compared to the control group ($p<0{,}05$) Table 42

The significance is greater when the combined incidence in men and women is taken into consideration ($p<0{,}01$)

Diabetes had presented before the age of 40 years in only one patient.

Comments

Ruikka et al (1966) found the incidence of diabetes in men and women to be 4 and 7 % respectively Kuusisto s total series of patients with infarction in Helsinki (Kuusisto et al 1960) contained a 6,2 % incidence of diabetes in men and 16 % incidence of women making a combinate incidence of 9 8 %. In Malmö Johansson (1972) found diabetes to be present in 7 6 % of men and 12 7 % women. In these series the proportion younger patients obviously lowers the dence of diabetes.

Summary

Diabetes is a known risk factor in patients and on the basis of the study it also appears to be a risk factor the elderly

3 Lipids

Serum cholesterol had been measured to infarction in only a small proportion patients Two men and 6 women were kno to have a high cholesterol level.

Serum cholesterol was measured about 8 d after infarction. The mean value in 1 examined male patients with infarction 5 77± 1,45 mmol/l and in female patients th mean was 6,42± 1 68 mmol/l (N.S.D) 6 and 10 women had a cholesterol level of ov 7 5 mmol/l. Serum cholesterol estimations wer obtained from 55 men and 57 women.

In the control group the mean cholesterol level in men was 7,09± 1 33 mmol/l and *women 7 54± 1 54 mmol/l.*

Comments

Serum cholesterol has been found to var with age Women over 60 years of age ha a significantly higher cholesterol level than men (*Lopez et al 1967 O'Neal 1974*) A definite decrease in the cholesterol level occurs in men after the age of 60 years, but this decrease does not occur in women un til after the age of 80 years (*O'Neal 1974*)

Following myocardial infarction a decrease in both free cholesterol and cholesterol esters has been noted and their mean value is lowest 8 days after infarction (*Ritland 1975*) 8 days after infarction cholesterol was 67 % of the normal *level and this was followed by a rise*

Table 42. Diabetes

	Patients with myocardial infarction			Controls		
	Males	Females	Total	Males	Females	Total
definite diabetes	20	33	53	2	7	9
not definite diabetes	2	1	3	0	0	0
no diabetes	82	85	167	36	54	89
unknown	4	14	18	0	2	2
	108	133	241	38	62	100

reaching 96 % of the normal level after 7 weeks.

1 Enger et al. (1970) found that the cholesterol level was 9 days after infarction about 10 % below the level 3 months after infarction.

The investigation was hampered by the fact that the cholesterol level was estimated during the period of convalescence in hospital when its level was approaching its lowest. In view of the fact that the majority of the serum cholesterol levels were estimated about 8 days after infarction, Ritland's (1975) calculation for the correction of this level was also performed. Mean cholesterol levels of 8,6 mmol/l for men and 9,5 mmol/l for women were obtained, and these levels are significantly higher than in the control group (p<0,001)

Summary On the basis of present study the high serum cholesterol level appeares possibly to be risk factor in the elderly

Triglycerides were investigated together with the serum cholesterol about 8 days after infarction. There was no information about levels prior to infarction in any patient

The mean level in men was 1,63± 0,8 mmol/l and in women 1,60± 0,63 mmol/l (N.S.D.) 4 men and 11 women had levels over 1 7 mmol/l.

In the control group the mean levels were 1,50± 0,5 mmol/l and 1,62± 0,69 mmol/l for men and women respectively There was no statistically significant difference between the patients and the controls.

Comments
According to Ritland (1975) the triglyceride level rises after infarction to achieve a maximum level of 6 % of the normal level after weeks This increase was not statistically significant however

Summary
No statistically significant difference was seen when compared to the control group The triglyceride level is thus probably not a significant risk factor

4. Physical inactivity
More than half of patients with myocardial infarction (55 men and 74 women, altogether 129 patients) had limited physical activity Only 28 (28 %) of the controls had limited physical activity Spare time activity was similar in both groups.

The fact that elderly patients have many illnesses which curtail the physical activity makes it difficult to draw conclusions about the significance of physical inactivity

5 Obesity
In this study the body mass index (i.e. Quitelet's index) was used in which the patients weight is divided height squared (Keys et al. 1972)

The controls were measured and weighed at the time of their examination. The patients with infarction had these measurements taken in hospital. Some of the information was obtained from the patients themselves.

The Quitelet's index in male patients was 2,39± 0,44 and in male controls 2,38± 0,74 (N.S.D.) *The index in female patients was* 2,45± 0,38 and in female controls 2,54± 0,39 (N.S.D.)

Summary
In this study body mass or overweight was not found to be associated with *myocardial* infarction Overweight would thus not seem to be a risk factor in the elderly

6 Smoking
There were 26 male (24,1 %) and 5 female smokers making a total of 31 patients (12,6 %) who smoked. Table 43 There were significantly more male smokers than female smokers (p<0,001) The control group showed the same tendency

However the patient group did not contain more smokers than the control group.

Table 43 Smoking

	Patients with myocardial infarction			Controls		
	Males	Females	Total	Males	Females	Total
smokers	26	5	31	6	2	8
has stopped smoking earlier	39	8	47	25	3	28
never smoked	31	92	123	7	56	63
unknown	12	28	40	0	1	1
	108	133	241	38	62	100

Summary
On the basis of this study smoking would not appear to be a significant risk factor in the elderly

22 OTHER PREVIOUS ILLNESSES

Many patients had a history of previous ill nesses prior to infarction.

Previous infarction
Table 44 shows the number of patients with previous infarction. There was no significant difference between the men in the patient group and the control group (N.S.D) The wom en in the patient group had significantly more previous infarctions ($p < 0{,}01$) The com bined total of men and women in the patient group also showed a significantly greater in cidence of previous infarction ($p < 0{,}01$)

The mean incidence of denite previous in farction in men was 1,2 and in women 1 1 Infarction had occurred on average 3,6 years previously in men and 2 5 years previously in women.

Comments
Definite previous infarction increased the risk of infarction in women but no difference between male patients and male controls was seen in this study

Angina of effort
Angina of effort occurring more than 28 days before the attack was present in 124 pa tients (52 %) In the control group only 25 % gave such history ($p < 0{,}001$)

In the patient group men had had angina pectoris 5,83± 5,9 years on average and women 5 8± 7,4 years. In the control group the symp tom had been present for 6,6± 7,0 years in men and 6 3± 5 9 years in women.

Comments
Puska et al (1974) reported an inciden angina of effort in 27 5 % of the men and % of the women in their series.

Stroke
11 men and 15 women had a hi of stroke prior to infarction, giving a tot 26 patients (10,8 %) Definite stroke had curred in 2 men and 4 women in the co group The difference between the patient control group is not statistically significant

Intermittent claudication
Men had a significantly greater incidence intermittent claudication than women (0,001) and also a significantly greater i dence than the controls ($p < 0\ 001$) The wom in the control group had a significantly grea *incidence of intermittent claudication* than t female patients ($p < 0\ 01$)

Previous gynecological surgery e.g. hyster tomy or ovarectomy did not appear to be significance in this series.

Other cardiovascular diseases
These were usually heart failure cor pulm nale or atrial fibrillation. The incidence these illnesses in men was 32,4 % (35 patie and in women 45 1% (60 patients) There no significant difference for the control grou

Role of previous illnesses in hospital mortali rate
Examination of the patients' previous illnes and the subsequent hospital mortality rate re veals that previous myocardial infarction dia betes stroke and heart failure appear to b associated with a high hospital mortality an apparently worsen the patient's prognosi Table 44

Table 44 Relationship of previous illnesses to hospital mortality

	Males	%	died	%	Females		died	
definite previous myocardial infarction	29	26 9	17	58,6	10	14,3	10	52,6
angina pectoris	61	56,5	19	31 1	63	47 4	10	30,2
hypertension	11	10,2	2	18,2	32	24 1	8	25 0
heart failure	16	14,8	6	37,5	46	34 0	23	50,0
atrial fibrillation	4	3 7	4	100 0	0	4,5	3	50,0
stroke	11	10,2	4	36 4	15	11,3	7	46,7
diabetes	22	20 4	9	40 9	34	25 6	23	67,6

23 FAMILIAR FACTORS

1 *Paternal age of death*

The mean age of death of male patients' fathers was 64,7± 13 7 years and female patients' fathers 66 1± 12,4 years. In the control group the mean age of paternal death was 67,0± 5 5 years for men and 65 1± 15 7 years for women

2 *Maternal age of death*

The mean age of maternal death in the case of male patients was 70 7± 14 3 years and in the case of female patients 70 5± 13 9 years. (N.S.D.)

Myocardial infarction in siblings did not appear to be of significance when compared to the control group. (N.S.D.)

3 Paternal myocardial infarction

Paternal myocardial infarction did not appear to be of any significance when compared to the control group

4 *Maternal myocardial infarction*

Maternal myocardial infarction had occurred in the case of 3 male patients and 22 female patients ($p < 0{,}00$). Maternal myocardial infarction seemed to increase the risk of myocardial infarction in women. Table 45

Summary

The information about paternal illnesses is extremely vague since the question concerned the parents of elderly patients many of whom lived at the end of the last century or the early part of this century. Information regarding siblings was more reliable. On the basis of the present study however it may be stated that maternal myocardial infarction may act as an additional risk indicator in women

24 RESULTS OF FOLLOW UP STUDY

1 *Attendance for check up*

Three months *after* infarction 66 women were asked to attend for a check up 6 of women failed to attend. Two of these were attending Turku City Hospital, one had moved away from the area and three other patients otherwise failed to turn up

54 men were asked to attend and 4 of these did not attend. Two of these patients were attending Turku City Hospital and two failed to turn up even though they were known to be still alive.

After one year 43 men were requested to attend for check-up and all but three attended. One of these men was attending Turku City Hospital and although both of the other two men were alive, they failed to show up despite receiving the appointment for a check up. One of the latter two men was in poor health but the other was active and working as a barrister

50 women were asked to attend for a check up and 46 showed up. One woman was attending Turku City Hospital and three others otherwise failed to turn up.

2 *Socio-economic factors after myocardial infarction*

The mode of residence remained unchanged after infarction and the number of patients in institutions actually decreased.

3 *Physical activity after infarction*

After three months 60 patients (54 5 /) were able to go shopping in the city and the number increased to 62 patients (72 /) after a year

There were 1 patients confined to bed before infarction. Three months after infarction

Table 45 Maternal myocardial infarction

	Patients			Controls		
	Males	Females	Total	Males	Females	Total
yes	3	22	25	3	4	7
no	56	41	97	25	37	62
unknown	49	70	119	10	21	31
	108	133	241	38	62	100

Table 46. Angina prectoris after infarction

	after 3 months				after one year		
	Males	Females	Total	o	Males	Females	Total
no angina pectoris	19(38%)	21(35 %)	40	37	20(50%)	13(28%)	33
occasional attacts of angina pectoris	16	17	33	30	12	10	22
attacks of 2—3 times a week	8	10	16	15	4	12	16
attacks every day or more often	9	11	20	18	4	10	14
unknown	0	1	1		0	1	1

there was only 1 patient confined to bed and after a year there were 3 patients

Summary

During the follow up period the patients were more active and there was a decrease in number of patients confined to bed This apparently results from the fact that the patients in poor condition died and those in satisfactory condition survived to lead an active life

4 Patient s ability to look after himself increased relatively during the follow-up period i e patients requiring assistance prior to in farction numbered 37 (15 %), after three months the number fell to 9 (8,2 %) and to 5 (5,2 %) after a year

5 Domestic help

There was an increased demand for domestic help After 3 months 9 women (8,2 %) requi red such a service and after a year figure was the same (9 women or 14 6 %)

The majority of patients looked after them selves after infarction.

6 Visits to the doctor

Visits to the doctor increased after in farction. After 3 months 68 patients (61,8 %) visited a *doctor and* after a year 69 patients (80,2 %) attended. There was no statistically significant difference between male and female attenders.

Chest pain after infarction

Table 46 reveals the incidence of chest p– After 3 months *19 male patients* (38 %) d not have chest pain and after a year the nu ber was 20 patients (50 %) During the follo up period chest pain was absent in 21 wom (35 %) after 3 months and in women (28 after a year

Summary

The number of men with chest pain decrea during the follow up while in women t number appeared to increase.

The amount of medication taken for attacl of chest pain also relected the incidence c character of chest pain. Many patients to nitroglycerine for attacks of chest pain or th *some corresponding medication. Nitroglycerir* caused headaches in some patients thus pr cluding further use of the drug.

After a period of 3 months the mean nu ber of nitroglycerine tablets taken per wee by men was 5,2± 21 1 and after a year th mean was 2,0± 4,8 tablets (N.S.D) Wome took 3 5± 5,4 nitroglycerine tablets after 3 months and 5 7± 12,0 tablets after a yea (N.S.D)

Functional classification after infarctio Three months following infarction 30 men (6 /) and 38 women (63 3 %) had functiona class of I—IV Table 47

Table 47 Functional classification after *myocardial infarction*

	after 3 months			after one year		
	Males	Females	Total	Males	Females	Total
Class I does not limit physical activity	7	7	14	5	7	12
Class II *a mild limitation*	12	16	28	13	15	28
Class III considerable limitation	9	14	23	4	8	12
Class IV symptoms at rest	2	1	3	1	1	2
no heart failure	20	21	41	17	14	31
unknown	0	1	1	0	1	1

After a year functional class I—IV was present in 23 men (57,7 %) and 31 women (67,4 %). No statistically significant difference was noted between men and women in the functional classification.

Orthopnoea after infarction was present in 5 men (10 %) and 12 women (20 %) 3 months after infarction. After a year the symptom was present in 2 men (4 %) and 8 women (13,3 %).

Lower limb oedema of cardiac origin was present in 4 men (8 %) and 12 women (20 %) 3 months following infarction and after a year oedema was present in 2 men (4 %) and 11 women (24 %).

On chest auscultation *basal crepitations* were audible in 6 men (12 %) and 5 women (8,3 %) after 3 months and in one man and one woman after a year.

Blood pressure
Systolic blood pressure (supine) was 145,5± 23,8 mmHg in men and 157,3± 24,3 mmHg in women 3 months after infarction. After a year it was 144,5± 24,8 mmHg in men and 158,1± 26,8 mmHg in women.

Diastolic blood pressure D₁ was 83,4± 13,3 mmHg in men and 85± 10,5 mmHg in women 3 months after infarction. After a year it was 80,6± 12,6 mmHg in men and 83,5± 10,6 mmHg in women.

D₅ was 78,4± 11,7 mmHg in men and 81,2± 10,0 mmHg in women 3 months following infarction and after a year it was 75,3± 12 mmHg in men and 77,6± 11,4 mmHg in women.

25 MINNESOTA CODE ON DISCHARGE FROM HOSPITAL AND DURING FOLLOW UP

Q and QS patterns were commonly present on discharge from hospital. They were present in 84 men (77,8 %) and 79 women (59,4 %). During the follow up the relative number decreased further. Table 48.

In the control group Q-wave changes were present in 6 men (15,8 %) and 7 women (11,3 %). Only 5 men in the control group were known to have had definite infarctions. Therefore the remaining one man and 7 women apparently had completely silent myocardial infarctions.

Table 48. Q and QS pattern in ECG on discharge from hospital and during follow-up.

	Males				Females			
Minnesota code	on discharge from hospital	3 months	one year	controls	on discharge from hospital	3 months	one year	controls
1-1-1	3	0	0	0	5	1	0	0
1-1-2	13	1	0	0	3	0	0	0
1-1-3	3	1	2	0	4	0	4	0
1-1-4	23	4	4	2	18	5	7	2
1-1-5	2	0	0	0	0	0	0	0
1-1-6	5	3	3	1	0	1	3	0
1-1-7	2	0	0	0	0	0	0	0
1-2-1	0	0	0	0	0	0	0	0
1-2-2	1	0	3	0	5	0	5	0
1-2-3	0	0	0	0	0	0	0	0
1-2-4	1	3	2	0	6	6	2	0
1-2-5	0	0	0	0	0	0	0	0
1-2-6	0	1	0	0	0	0	0	0
1-2-7	4	0	0	0	2	1	0	0
1-2-8	19	8	2	1	23	6	4	2
1-3-1	0	0	0	0	0	0	0	0
1-3-2	1	3	2	0	5	1	0	2
1-3-3	4	4	0	1	3	7	2	1
1-3-4	1	1	1	1	0	2	1	2
1-3-5	0	0	0	0	0	0	0	0
1-3-6	12	0	0	0	5	0	0	0
	84	29	19	6	70	29	24	7
no changes	14	21	22	32	50	30	25	53

Table 46 Angina prectoris after infarction

	after 3 months				after one year		
	Males	Females	Total		Males	Females	Total
no angina pectoris	19(38%)	21(35%)	40	37	20(50%)	13(28°)	33
occasional attacts of angina pectoris	16	17	33	30	12	10	22
attacks of 2—3 times a week	6	10	16	15	4	12	16
attacks every day or more often	9	11	20	18	4	10	14
unknown	0	1	1		0	1	1

there was only 1 patient confined to bed and after a year there were 3 patients.

Summary
During the follow up period the patients were more active and there was a decrease in number of patients confined to bed. This apparently results from the fact that the patients in poor condition died and those in satisfactory condition survived to lead an cctive life.

4 Patient s ability to look after himself
increased relatively during the follow-up period i.e patients requiring assistance prior to in farction numbered 37 (15 %), after three months the number fell to 9 (8,2 %) and to 5 (5,2 %) after a year

5 Domestic help
There was an increased demand for domestic help After 3 months 9 women (8,2 %) requi red such a service and after a year figure was the same (9 women or 14 6 %)

The majority of patients looked after them selves after infarction.

6 Visits to the doctor
Visits to the doctor increased after in farction. After 3 months 68 patients (61,8 %) visited a doctor and after a year 69 patients (80,2 %) attended. There was no statistically significant difference between male and female attenders

Chest pain after infarction
Table 46 reveals the incidence of chest After 3 months 19 male patients (38 %) di not have chest pain and after a year the ber was 20 patients (50 %) During the follow up period chest pain was absent in 21 w (35 %) after 3 months and in women (28 % after a year

Summary
The number of men with chest pain decrea during the follow up while in women the number appeared to increase.

The amount of medication taken for attacks of chest pain also relected the incidence character of chest pain. Many patients took nitroglycerine for attacks of chest pain or some corresponding medication. Nitroglycerine caused headaches in some patients thus pre cluding further use of the drug.

After a period of 3 months the mean num ber of nitroglycerine tablets taken per week by men was 5 2± 21 1 and after a year the mean was 2,0± 4 8 tablets (N.S D) Women took 3 5± 5,4 nitroglycerine tablets after 3 months and 5 7± 12,0 tablets after a year (N.S.D)

Functional classification after infarction
Three months following infarction 30 men (60 %) and 38 women (63 3 %) had functional class of I—IV Table 47

Table 47 Functional classification after myocardial infarction

	after 3 months			after one year		
	Males	Females	Total	Males	Females	Total
Class I does not limit physical activity	7	7	14	5	7	12
Class II a mild limitation	12	10	28	13	15	28
Class III considerable limitation	9	14	23	4	8	12
Class IV symptoms at rest	2	1	3	1	1	2
no heart failure	20	21	41	17	14	31
unknown	0	1	1	0	1	1

After a year functional class I—IV was present in 23 men (57 7 %) and 31 women (67,4 %) No statistically significant difference was noted between men and women in the functional classification.

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Lower limb oedema of cardiac origin was present in 4 men (8 %) and 12 women (20 %) 3 months following infarction and after a year oedema was present in 2 men (45) and 11 women (24 %)

On chest auscultation basal *crepitations* were audible in 6 men (2 %) and 5 women (8 3 %) after 3 months nd in one man and one woman after year

Blood pressure
Systolic blood pressure (supine) was 145 5 $\pm$ 23,8 mmHg in men and 157 3 $\pm$ 24 3 mmHg in women 3 months after infarction. After a year it was 144 5 $\pm$ 24,8 mmHg in men and 158 1 $\pm$ 26,8 mmHg in women

Diastolic blood pressure D_4 was 83,4 $\pm$ 13 3 mmHg in men and 85 $\pm$ 10,5 mmHg in women 3 months after infarction. After a year it was 80,6 $\pm$ 12,6 mmHg in men and 83 5 $\pm$ 10,6 mmHg in women.

D_5 was 78,4 $\pm$ 11 7 mmHg in men and 81,2 $\pm$ 10,0 mmHg in women 3 months following infarction and after a year it was 75 3 $\pm$ 12 mmHg in men and 77,6 $\pm$ 11,4 mmHg in women.

25 MINNESOTA CODE ON DISCHARGE FROM HOSPITAL AND DURING FOLLOW UP

Q and QS *patterns* were commonly present on discharge from hospital. They were present in 84 men (77,8 %) and 79 women (59,4 %). During the follow up the relative number decreased further T ble 48

In the control group Q-wave changes were present in 6 men (15,8 %) and 7 women (13 %) Only 5 men in the control group were known to have had definite infarctions. Therefore the remaining one man and 7 women apparently had completely silent myocardial infarctions.

Table 48 Q and QS pattern in ECG on discharge from hospital and during follow-up

	Males				Females			
Minnesota code	on discharge from hospit l	3 months	one year	controls	on discharge from hospital	3 months	one year	controls
1–1 1	3	0	0	0	5	1	0	0
1–1 2	13	1	0	0	3	0	0	0
1 1–3	3	1	2	0	4	0	4	0
1–1 4	23	4	4	2	18	5	7	2
1–1–5	2	0	0	0	0	0	0	0
1–1 6	5	7	3	1	0	1	3	0
1 1 7	2	0	0	0	0	0	0	0
1 2–1	0	0	0	0	0	0	0	0
1 2 2	1	0	3	0	5	0	1	0
1 2 3	0	0	0	0	0	0	0	0
1 2–4	1	3	2	0	6	6	2	0
1 2 5	0	0	0	0	0	0	0	0
1 2 6	0	1	0	0	0	0	0	0
1 2 7	4	0	0	0	2	1	0	0
1 2 8	19	9	2	1	23	6	4	2
1–3 1	0	0	0	0	0	0	0	0
1–3 2	1	3	2	0	5	1	0	2
1 3–3	4	4	0	1	3	7	2	1
1–3 4	1	1	1	1	0	2	1	2
1–3 5	0	0	0	0	0	0	0	0
1 3 6	12	0	0	0	5	0	0	0
	84	29	19	6	79	29	24	7
no changes	14	21	22	32	30	30	25	53

Table 49 QRS deviation

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: con
2—1	39	19	18	15	42	12	11	14
2—2	1	0	0	0	1	1	0	4
2—3	2	1	1	1	0	0	0	3
2—4	0	0	0	0	1	0	1	4
2—5	45	24	19	15	72	44	33	41

Table 50 High amplitude R waves

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: contr
no	87	43	35	35	112	47	38	56
3—1	8	6	4	3	8	7	6	6
3—2	0	0	0	0	1	2	0	0
3—3	4	0	1	0	5	3	2	0
unknown	9	1	0	0	7	1	0	0

Table 51 S—T junction (J) and segment depression

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 years	Females: controls
no	33	16	22	34	30	10	15	49
4—1	39	14	8	0	71	30	15	6
4—2	10	14	5	3	11	12	9	4
4—3	16	5	5	0	9	7	7	3
4—4	1	1	0	0	0	0	0	0

Table 52 T wave items

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
no	33	15	21	34	30	8	12	47
5—1	33	8	1	0	25	5	3	2
5—2	33	24	15	0	63	43	28	13
5—3	0	1	3	1	1	2	4	0
5—4	0	1	0	0	2	1	0	0

During the follow up the Q vawe changes decreased in both men and women After 3 months they were present in 29 men (58 %) and 29 women (48.3 %) After a year changes were present in 19 men (47.5 %) and 24 women (52,2 %)

Comments
Sourander et al. (1967) found Q wave changes in 16 men (8.3 %) and 8 women (4.1 %) out of a total of 481 people aged over 65 years. This is slightly less than in the control group of the present study

Summary
During the follow-up the Q-wave changes decreased perhaps mainly therefore that patients died during this time.

QRS-axis deviation
Table 49 reveals that during the follow-up left deviation of axis increased in men and decreased in women.
High amplitude R waves. Table 50.

ST segment depression and T-waves items, decreased in both sexes during the follow-up. Table 51—52.

A—V conduction defects
2 men and 5 women had first degree A—V block on discharge from hospital. These changes decreased to the extent that after 3 months they were present in only one man and after a year in only one woman. Table 53

Ventricular conduction defects
Table 54 shows that both LBBB and RBBB diminished markedly during the follow up period. Table 55 reveals that LAHB and LPHB occurred approximately to the same extent in both the patient and the control group. Bifascicular block was not found in the control group but was present in 2 men and one woman on discharge from hospital. These patients remained alive throughout the follow-up period.

Comments
Beck et al. (1974) discovered LAHB in 55 patients (9,05 %) in their series of 609 patients. It occurred particularly as a compli-

Table 53 A—V conduction defects

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
nn	96	48	40	38	122	50	45	61
6—1	1	0	0	0	0	0	0	0
6—2	0	0	0	0	0	0	0	0
6—3	2	1	0	0	5	0	1	1
6—4	0	0	0	0	0	0	0	0
6—8	0	0	0	0	0	0	0	0

Table 54 Ventricular conduction defects

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
nn	56	42	30	28	84	51	38	48
7—1	7	1	1	1	6	1	0	3
7—2	10	1	1	6	6	3	4	2
7—3	0	0	0	0	0	0	0	0
7—4	0	1	1	0	0	0	0	0
7—5	1	0	0	0	0	0	0	0
7—6	12	4	7	3	11	4	4	8

Table 55 Differentiation of ventricular conduction defects

	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
LAH	5(4,6 %)	3	5	2(5,3 %)	9(8,7 %)	1	3	3(4,8 %)
LPH	7(6,5 %)	1	2	1(2,7 %)	2(1,5 %)	3	1	4(6,5 %)
RBBB+LAH	2(1,9 %)	2	2	0	1	1	1	0
RBBB+LPH	0	0	0	0	0	0	0	0

Table 49 QRS deviation

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	contro.
2–1	39	19	18	15	42	12	11	14
2–2	1	0	0	0	1	1	0	0
2–3	2	1	1	1	0	0	0	5
2–4	0	0	0	0	1	0	1	0
2–5	45	24	19	15	72	44	33	40

Table 50 High amplitude R waves

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	controls
no	87	43	35	35	112	47	38	56
3–1	8	6	4	3	8	7	6	6
3–2	0	0	0	0	1	2	0	0
3–3	4	0	1	0	5	3	2	0
unknown	9	1	0	0	7	1	0	0

Table 51 S–T junction (J) and segment depression

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 years	controls
no	33	16	22	34	36	10	15	49
4–1	39	14	8	0	71	30	15	6
4–2	10	14	5	3	11	12	9	4
4–3	16	5	5	0	9	7	7	3
4–4	1	1	0	0	0	0	0	0

Table 52 T wave items

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	controls
no	33	15	21	34	36	8	12	47
5–1	33	8	1	0	25	5	3	2
5–2	33	24	15	0	63	43	28	13
5–3	0	1	3	1	1	2	4	0
5–4	0	1	0	0	2	1	0	0

During the follow up the Q-vawe changes decreased in both men and women. After 3 months they were present in 29 men (58 %) and 29 women (48.3 %). After a year changes were present in 19 men (47.5 %) and 24 women (52,2 %).

Comment

Sourander et al (1967) found Q-wave changes in 16 men (8.3 %) and 8 women (4.1 %) out of a total of 481 people aged over 65 years. This is slightly less than in the control group of the present study.

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During the follow up the Q-wave changes de creased perhaps mainly therefore that patients died during this time.

QRS *axis deviation*

Table 49 reveals that during the follow-up left deviation of axis increased in men and decreased in women.

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ST segment depression and T waves, items decreased in both sexes during the follow up Table 51—52.

A—V conduction defects

2 men and 5 women had first degree A—V block on discharge from hospital. These changes decreased to the extent that after 3 months they were present in only one man and after a year in only one woman. Table 53

Ventricular conduction defects

Table 54 shows that both LBBB and RBBB diminished markedly during the follow up period. Table 55 reveals that LAHB and LPHB occurred approximately to the same extent in both the patient and in the control group Bifascicular block was not found in the control group but was present in 2 men and one woman on discharge from hospital. These patients remained alive throughout the follow-up period.

Comments

Beck et al. (1974) discovered LAHB in 55 patients (9,05 %) in their series of 609 patients. It occurred particularly as a compli-

Table 53. A—V conduction defects

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 yea	controls
no	66	48	40	38	122	59	45	61
6—1	1	0	0	0	0	0	0	0
6—2	0	0	0	0	0	0	0	0
6—3	2	1	0	0	0	0	0	0
6—4	0	0	0	0	5	0	1	1
6—5	0	0	0	0	0	0	0	0

Table 54. Ventricular conduction defects

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
no	56	42	30	28	64	51	38	48
7—1	7	1	1	1	6	1	0	3
7—2	10	1	1	6	6	3	4	2
7—3	0	0	0	0	0	0	0	0
7—4	0	1	1	0	0	0	0	0
7—5	0	0	0	0	0	0	0	0
7—6	12	4	7	3	11	4	4	8

Table 55. Differentiation of ventricular conduction defects

	Males				Females			
	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
LAH	5(4,6%)	3	5	2(5,3%)	9(6,7%)	1	3	3(4,8%)
LPH	7(6,5%)	1	2	1(2,7%)	2(1,5%)	3	1	4(6,5%)
RBBB+LAH	2(1,9%)	2	2	0	1	1	1	0
RBBB+LPH	0	0	0	0	0	0	0	0

Table 49 QRS deviation

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	control
2—1	39	19	18	15	42	12	11	14
2—2	1	0	0	0	1	1	0	0
2—3	2	1	1	1	0	0	0	5
2—4	0	0	0	0	1	0	1	0
2—5	45	24	19	15	72	44	33	40
—								

Table 50 High amplitude R waves

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	controls
no	87	43	35	35	112	47	38	56
3—1	8	6	4	3	8	7	6	6
3—2	0	0	0	0	1	2	0	0
3—3	4	0	1	0	5	3	2	0
unknown	9	1	0	0	7	1	0	0

Table 51 S—T junction (J) and segment depression

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 years	controls
no	33	16	22	34	36	10	15	49
4—1	39	14	8	0	71	30	15	6
4—2	10	14	5	3	11	12	9	4
4—3	16	5	5	0	9	7	7	3
4—4	1	1	0	0	0	0	0	0

Table 52. T wave items

Minnesota code	Males: on discharge from hospital	after 3 months	after 1 year	controls	Females: on discharge from hospital	after 3 months	after 1 year	controls
no	33	15	21	34	36	8	12	47
5—1	33	8	1	0	25	5	3	2
5—2	33	24	15	0	63	43	29	13
5—3	0	1	3	1	1	2	4	0
5—4	0	1	0	0	2	1	0	0

During the follow up the Q-vawe changes decreased in both men and women. After 3 months they were present in 29 men (58 %) and 29 women (48.3 %). After a year changes were present in 19 men (47.5 %) and 24 women (52,2 %).

Comments

Sourander et al (1967) found Q-wave changes in 16 men (8.3 %) and 8 women (4.1 %) out of a total of 481 people aged over 65 years. This is slightly less than in the control group of the present study.

Summary

During the follow-up the Q-wave changes decreased perhaps mainly therefore that patients died during this time.

QRS-axis deviation
Table 49 reveals that during the follow-up left deviation of axis increased in men and decreased in women.
High amplitude R waves. Table 50.

ST segment depression and T waves, items decreased in both sexes during the follow-up T ble 51—52.

A—V conduction defects
2 men and 5 women had first degree A—V block on discharge from hospital. These changes decreased to the extent that after 3 months they were present in only one man and after a year in only one woman. Table 53

Ventricular conduction defects
Table 54 shows that both LBBB and RBBB diminished markedly during the follow-up period. Table 55 reveals that LAHB and LPHB occurred approximately to the same extent in both the patient and in the control group. Bifascicular block was not found in the control group but was present in 2 men and one woman on d scharge from hospital. These patients remained alive throughout the follow-up period.

Comments
Beck et al. (1974) discovered LAHB in 55 patients (9,05 %) in their series of 609 patients. It occurred particularly as a compli-

Table 53. A—V conduction defects

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
no	90	48	40	38	122	59	45	61
6–1	1	0	0	0	0	0	0	0
6–2	0	0	0	0	0	0	0	0
6–3	2	1	0	0	5	0	1	1
6–4	0	0	0	0	0	0	0	0
6–5	0	0	0	0	0	0	0	0

Table 54. Ventricular conduction defects

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
no	56	42	30	28	64	51	38	48
7 1	7	1	1	1	6	1	0	3
7 2	10	1	1	6	6	3	4	2
7 3	0	0	0	0	0	0	0	0
7 –4	0	1	1	0	0	0	0	0
7 5	0	0	0	0	0	0	0	0
7 6	12	4	7	3	11	4	4	8

Table 55 Differentiation of ventricular conduction defects

	Males				Females			
	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
LAH	6(4,6%)	3	5	2(5,3%)	9(6,7%)	1	3	3(4,6%)
LPH	7(6,5%)	1	2	1(2,7%)	2(1,5%)	3	1	4(6,3%)
RBBB+LAH	2(1,9%)	2	2	0	1	1	1	0
RBBB+LPH	0	0	0	0	0	0	0	0

Table 49 *QRS deviation*

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
2—1	39	19	18	15	42	12	11	14
2—2	1	0	0	0	1	1	0	0
2—3	2	1	1	1	0	0	0	5
2—4	0	0	0	0	1	0	1	0
2—5	45	24	19	15	72	44	33	40

Table 50 High amplitude R waves

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
no	87	43	35	35	112	47	38	36
3—1	8	6	4	3	8	7	6	6
3—2	0	0	0	0	1	2	0	0
3—3	4	0	1	0	5	3	2	0
unknown	9	1	0	0	7	1	0	0

Table 51 *S—T junction (J) and segment depression*

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 years	Females: controls
no	33	16	22	34	36	10	15	49
4—1	39	14	8	0	71	30	15	6
4—2	10	14	5	3	11	12	9	4
4—3	16	5	5	0	9	7	7	3
4—4	1	1	0	0	0	0	0	0

Table 52. T wave items

Minnesota code	Males: on discharge from hospital	Males: after 3 months	Males: after 1 year	Males: controls	Females: on discharge from hospital	Females: after 3 months	Females: after 1 year	Females: controls
no	33	15	21	34	36	8	12	47
5—1	33	8	1	0	25	5	3	2
5—2	33	24	15	0	63	43	29	13
5—3	0	1	3	1	1	2	4	0
5—4	0	1	0	0	2	1	0	0

During the *follow up* the *Q-vawe changes* decreased in both men and women. After 3 months they were present in 29 men (58 %) and 29 women (48.3 %). After a year changes were present in 19 men (47.5 %) and 24 women (52.2 %).

Comments

Sourander et al (1967) found Q wave changes in 16 men (8.3 %) and 8 women (4.1 %) out of a total of 481 people aged over 65 years. This is slightly less than in the control group of the present study

omments
ourander et al. (1967) found arrhythmias n 48 men (25,4 %) and 46 women (22,2 %) n their series, extrasystoles were present in 8 men and 17 women and atrial fibrillation n 16 men and 18 women.

Miscellaneous items
The great majority of these consisted of ST segment elevation in the chest leads in particular These changes also diminished markedly during the follow-up Table 37

Comments
Sourander et al. (1967) reported miscellaneous items in 44 men (23,9 /) and 23 women (11 7 %)

26 THE QRS-AXIS IN THE ECG

Three months after infaction the QRS-axis was +9 1 in men and 16,2 in women and +3 5 in men and +7,0 in women after a year.

cation of anterior infarction, was often transient and was twice as common in men as in women. The complication was not associ ated with an increased risk of mortality or risk of developing A—V-conduction disturbances but was associated with asystole and a danger of sudden death. The incidence of heart failure was greater in these patients than in those without conduction disturbances. There was no evidence of an increased risk of developing arrhythmias in these patients

Col et al. (1972) have investigated intraventri cular conduction defects occurring in 212 con secutive patients in a coronary care unit. The incidence of such defects was 24 % LAHB was most common (9,4 %) followed by in complete bilateral block (7 5 %) of which more than half was due to RBBB+LAHB Com plete RBBB and LBBB were more rare LPHB alone was rare. The hospital mortality rate for whole series was 21,2 % while the hospital mortality rate for patients with conduction de-fects was significantly greater (47 %) LAHB (25 %) was the least dangerous disturbance with regard to the hospital mortality rate.

Kalliomäki et al (1960) studied the associa-tion between myocardial infarction and bundle branch block (BBB) They found LBBB in 60 % of patients and RBBB in 40 % of patients. BBB was transitient in 5 % of patients. They found the higher mortality rate in patients with LBBB and myocardial infarction (32 1 %) than in pa-tients without conduction disturbances (16,2 %)

Arrhythmias
A large number of arrhythmias present on discharge from hospital were due to atrial fibrillation and during the follow up the number diminished markedly None of the male controls had atrial fibrillation. Atrial fib-rillation was present in approximately the same extent in female controls as in female pa tients after 3 months and one year The amount of frequent extrasystoles also decreased during the follow up. Table 56

Table 56 Arrhytmias

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
no	82	42	34	36	99	52	44	54
8—0	2	0	1	0	0	0	1	0
8—1	2	2	4	2	4	2	1	3(3%)
8—2	2	0	0	0	2	0	0	0
8—3	11 (11 1%)	2	2	0	21 (17,2%)	5	3	5(5%)
8—4	0	1	0	0	1	0	0	0
8—5	0	0	0	0	0	0	0	0
8—6	0	0	0	0	0	0	0	0
8—7	0	0	0	0	0	0	0	0
8—8	0	0	0	0	0	0	0	0
8—9	0	2	1	0	0	0	0	0
Arrhytmias total	17 (17%)			2 (2%)	28 (22%)			8(8%)

Table 57 Miscellaneous items at rest

	Males				Females			
Minnesota code	on discharge from hospital	after 3 months	after 1 year	controls	on discharge from hospital	after 3 months	after 1 year	controls
no	65	34	26	36	93	43	33	58
9—0	0	2	0	0	0	0	1	0
9—1	1	0	0	0	0	1	0	3
9—2	28	10	6	1	27	5	9	1
9—3	0	0	0	1	0	0	0	1
9—4—1	12	0	7	0	10	1	2	0
9—4—2	11	5	7	0	10	4	6	0
9—5	1	3	0	0	1	0	0	0
9—8	0	0	0	0	0	0	0	1

Comments
Sourander et al. (1967) found arrhythmias in 48 men (25,4 %) and 46 women (22,2 %). In their series, extrasystoles were present in 18 men and 17 women and atrial fibrillation in 16 men and 18 women.

Miscellaneous items
The great majority of these consisted of ST segment elevation in the chest leads in particular. These changes also diminished markedly during the follow-up. Table 57.

Comments
Sourander et al. (1967) reported miscellaneous items in 44 men (23,9 %) and 23 women (11,7 %).

26 THE QRS-AXIS IN THE ECG

Three months after infarction the QRS-axis was +9,1° in men and 16,2° in women and +3,5° in men and +7,0° in women after a year.



V
DISCUSSION

The incidence of myocardial infarction in the elderly

The incidence in Turku in patients under 75 years is clearly lower than in North Karelia but in the 75—79 year age group the incidence in Turku exceeds that in North Karelia. The incidence in women is the same but in the men the incidence is statistically higher in Turku. The incidence curve reveals that women, on average developed myocardial infarction about 10 years later than men.

Mortality

The total mortality was very high. 47.3 % of men and 37.5 % of women died suddenly at home or during the transport to hospital.

Hospital mortality was 38,2 % and it was found to increase with age.

After one year the cumulative mortality was 145 men (77.1 %) and 51 women (75,6 %).

The mortality in CCU was significantly lower both in men and in women than in ordinary medical ward. According this study it is impossible to conclude that the treatment in the CCU decreased the mortality. It would seem, however that the prompt detection of arrhythmias and conduction disturbances and their treatment in CCU could possibly give a better prognosis also in the elderly.

Exertion and stress preceding infarction

Myocardial infarction followed marked exertion or stress in only 20 patients. A surgical operation or other procedure preceded infarction in 0 patients. The significance of these factors as regards the number involved does not seem to be great but they may be significant in an individual case. 15 patients developed infarction after a stroke. This is possibly due to the fact that stroke is a serious strain for the patient and myocardial infarction then occurs during the period of recovery and rehabilitation. Another possibility is that it is sign of advanced generalized sclerosis in both the coronary arteries and the cerebral arteries.

The initial symptoms

of myocardial infarction in the elderly were different when they were compared to those in patients aged under 65 years. Chest pain was found to occur significantly more commonly in the younger patients. Elderly patients had significantly more other symptoms e.g. dyspnoea, vertigo and loss of consciousness than younger patients. Thus myocardial infarction apparently may have a more variable presentation in the elderly with fewer typical presenting symptoms.

This investigation also revealed the hospital mortality associated with various presenting symptoms and it may be observed that myocardial infarction presenting with chest pain had a better prognosis (hospital mortality rate 28,6 %) than, for example infarction presenting with loss of consciousness (59,1 %) or dyspnoea (57.1 %). When the chest pain lasted for a shorter time, for example, less than 6 hours the prognosis was better.

A surprising finding was that completely painless myocardial infarction was associated with a high hospital mortality (64,9 %). Painless myocardial infarction occurred primarily in patients suffering from diabetes, heart failure or chronic bronchitis. These patients usually developed acute dyspnoea but did not have any pain whatsoever. The patients also became weak and extremely ill-looking. The next most common presentation was loss of consciousness and then vertigo and weakness.

Why is infarction painless? Diabetics may have abnormalities of pain conduction or of the sensory nerves. Confusional patients can not express the chest pain.

The fact that patients with heart failure and chronic bronchitis often become accustomed

VI
GENERAL SUMMARY

During the period 1 3 1972—30.4. 1973 all patients from the city of Turku aged over 65 years with acute myocardial infarction were followed by Turku Heart Register according to the recommendations of WHO 101 men and 125 women who were clinically examined had altogether 241 acute episodes of myocardial infarction. Clinical examination was not possible in 87 men and 76 women, who died suddenly at home or on the way to hospital. A total of 404 cases of myocardial infarction was analyzed

From the beginning of this study age and sex-matched controls to 27 consecutive patients were selected by computer from the population registers of Turku 00 controls (38 men and 6 women) from a total of 127 were examined. The average age of patients and controls was the same as well as sex distribution. In order to compare the presenting symptoms in the elderly patients with the presenting symptoms in younger patients an additional control group was used.

The incidence of myocardial infarction in total population of Turku was highest in men in the age group 80—84 years 6 ,8 per 1000 per year and in women in the age group 85—89 years, 28 per 000 per year The incidence curve of women follows about 10 years behind the incidence curve of men

At home or during the transport to hospital 87 men (47 3 /) and 76 women (37,8 /o) died Hospital mortality was 38,2 / and it was found to increase with age

Post-mortem examination was performed in 77 % of clinically examined patients who died in the hospital after infarction.

20 patients had marked exertion or stress prior to infarction Infarction was preceded by infection in 14 patients and by stroke in 15 patients Infarction occurred in 7 patients after operation and in three following a major diagnostic or therapeutic procedure.

Symptoms

The most common presenting symptom was acute chest pain which was present in 157 patients (65 %) Other common symptoms were dyspnoea in 49 patients (20 /) loss of conciousness in 22 patients (9 /) vertigo and weakness in 7 (7 /o) recurrent vomiting in 17 (7 /) and confusional state in 12 patients (5 /o)

57 patients (23 /) had painless infarction and this phenomen occurred in patients with heart failure diabetes and chronic bronchitis.

Chest pain lasted for less than 6 hours in 10 patients (46 /) 6—12 hours in 31 patients 12—24 hours in 9 patients, and for more than 24 hours in 10 patients.

It was found that patients with chest pain made a better recovery from infarction than patients with painless infarction. The hospital mortality for patients with chest pain was 24 /o and for those without chest pain 65 /o.

Infarction was transmural in 160 cases (66 /) and subendocardial in 48 cases (20 %) the majority of infarctions occurred in the area of the anterior wall (134 cases or 56 /o) on the basis of ECG There were 47 (20 /o) infarctions in inferior wall.

Complications

Ventricular fibrillation occurred in 16 patients (6 7 /) and primary treatment was successfull in 8 (50) of these cases. Asystole was found in 31 patients. Of these long-term survival was achieved only in the case of one woman and all the others died. Pulmonary oedema was present in 39 patients (16,2 /o) and 8 (46,1 /) of these died. 18 patients (4,6 %) had ventricular tachycardia and 11 (61 1 /o) of these died. Atrial fibrillation was found in 20 patients (8 3 /) and 8 died (40 /o) The hospital mortality rate was 3 % for men and 39 1 / for women. After 3 months 46 5 / of the men and 47,2 % of the

to the sensation of continous dyspnoea may possibly be of significance with the result that the patient only experiences dyspnoea at the onset of infarction.

There were *numerous complications* in hospital and these were associated with a high mortality rate but it may be possible to treat conduction disturbances and serious arrhythmias including ventricular fibrillation and with the appropiate therapy the patient can recover The patient then may continue to lead a full life for years In other words if the opportunity for active treatment arises this treatment should be carried out also in the elderly

Bed rest
Bed rest for 6 days appears mostly to be sufficient in uncomplicated cases After this period the patients were allowed to sit out for 3 days and ambulation began cautiously on the 9th—10th day The mortality was greater when ambulation began later This is however an indication of complicated infarction

In Johansson's series in Malmö (Johansson 1972) sitting out and walking were started later and the mortality was the same in elderly patients and therefore there appears possibly to be no cogent reason for keeping elderly patients in bed for a long time

In practice the patient's general condition gives an indication of when mobilization can be started Many patients with dementia and loss of memory went to the toilet relatively soon after infarction in spite of the fact that they had not recovered from infarction and this was allowed

Risk factors
The role of classical risk factors in the elderly is also interesting. The role of risk factors was investigated in 241 clinically examined patients in this study Of the total material 163 patients died before proper clinical examination could be performed and the role of risk factors in these cases could not be ascertained.

On the basis of this study only diabetes and a high serum cholesterol appeared to be significant risk factors. The role of physical inactivity is difficult to assess in the elderly The question arises of whether patients with other risk factors for example hypertension, had already died before reaching the age of 65 years. This study could not clarify this problem.

Frequenting of the Finnish sauna does not appear at any rate to increase the amount of myocardial infarction.

The follow up
During the follow up period the patients' health appeared to show a relative improvement only with respect to physical activity and their ability to look after themselves. Chest pain diminished in men during the follow up but increased in women. The improvement in health during the follow up may be due to the fact that the patients in poor health died and were eliminated from the study and the fittest patients survived.

In this study the follow up was restricted to one year after which period 145 men (77.1 %) and 151 women (75.6 %) had died. Björck et al. (1958) found that in a 5 year follow up survivors from infarction had a longer survival rate than younger patients when the statistically expected mortality probabilities are taken into account.

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I wish to express my deep gratitude to Professor I. Rulkka M.D the Head of Turku City Hospital, for providing me with the facilities of his department. His extensiv clinical knowledge and his inspiring encouragemant were ery important to me during the study

My warm thanks are due Docent L Souran der M D who troughout the study gave me many valuable advice and encouraged me in many ways

I wish to thank Dr R Manner who gave me greatful assistance in the tudy of roentge nological changes expecially by using the valuable method of Turner

I wish to express my deep gratitude to Docent M. Arstila, M D who as the Head of Turku Heart Register with his extensive car diological knowledge always helped and en couraged me greatfully during the study

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Vilho Kontu

women had died and after a year the figures were 57,4 % for men and 60 % for women

Previous illnesses in particular previous myocardial infarction diabetes and heart failure worsened the prognosis.

The length of hospital treatment
The patients were treated in an ordinary medical ward for 12—14 days. 36 men (33 3 %) and 45 women (33 8 %) died in the medical ward. A number of patients were treated in the coronary care unit but the duration of therapy there was shorter The male mortality in the coronary care unit was 4 (15 7 %) and the female mortality was 7 (18 7 %)

The length of bed rest was 5 days on average and ambulation was started about 8 days after the beginning of the infarction. Patients who were allowed to walk 9 or more days after the start of the infarction had a worse prognosis in other words *patients in poor condition* were not allowed to walk till later and the mortality was greater because of their poor general condition.

The heart size was studied radiologically in 111 consecutive patients and in men it was 631± 211 ccm/m² and in women 536± 123 ccm/m² The pulmonary venous congestion was normal after infarction significantly more often in women than in men. The pulmonary venous congestion was classified according to Turner's method The same method was used to predict the pulmonary arterial pressure. The mean predicted pressure in men was 51± 23 mmHg and in women 47± 21 mmHg. Aortic calcification was more common in women.

At post mortem examination sclerosis was *found to be distributed evenly among the different* coronary arteries Infarction occurred as frequently in the antero-septal wall as in the *postero-lateral wall* Recent thrombus or occlusion was found most often in the right coronary artery and in the region of the descending branch of the left coronary artery and in the region of the left circumflex branch. Ventricular rupture was found in 8 patients (11 %) which is a very high figure compared to other series

The follow up study performed 3 months and 1 year after infarction revealed that the number of mobile patients increased and the number of bed ridden patients decreased. During the follow up chest pain in men appeared to decrease while in women it increased relatively

Risk factors Of the classical risk factors only diabetes and th high serum cholesterol level were found to be of significance in the elderly The role of physical inactivity was difficult to asses because the patients had to restrict their activity because *of various other illnesses.*

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My warm thanks are due Docent L Souranderr MD who troughout the study gave me many valuable advice and encouraged me in many ways

I wish to thank Dr R Manner who gave me greatful assistance in the study of roentgenological changes especially by using the aluable method of Turner

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Vilho Konu

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Medical Department A has its main competence in three different fields of internal medicine cardiology gastroenterology and nephrology The articles presented here, some of which are reviews, cover these three fields. A considerable part of the research activity in the department is of a theoretical nature and thus not particularly suited for presentation in Acta Medica Scandinavica. These topics are therefore not included in the present volume.

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Ole Jacob Broch — 70 years



On the 7th of July this year professor doctor medicinae, Ole Jacob Broch leaves his chair at the Medical School of the University of Bergen and the position as Head of the Medical Department A at the University Clinic. Except for a five years period he has served the Medical Faculty in Bergen as a professor since its start in 1948

He was prominent among those who carried the burdens in the early Gründer period of the University of Bergen in the fifties, and served as the Dean of the Faculty in the first part of the sixties when the Medical Faculty consolidated itself, being for the first time able to recruit its leading members from the University staff. He played a leading part in giving the dicipline of internal medicine the new image of subspecialities in the growth period of the late sixties, and has with success exercised the difficult art of functional expansion in the — in many ways not unhealthy — phase of zero resource growth and reevaluation of priorities in the seventies.

As an administrator he possesses the admirable quality of being ahead of his time. His recent proposal of adopting a board with an elected executive director for the administration of Medical Department A at the University Clinic was turned down Undoubtly this administrative structure is the most appropriate one for a department of internal medicine of subspecialities, and necessity will force its adoption in the future. He will be proved right once more. In the every day running of the clinic he has adopted administration by exception, giving his fellow workers a free hand. He could do this without risk, because his personality made him the self evident

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Per Lund-Johansen

meer studies in man

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However a few patients in the older stud es had a high cardiac output and a total peripheral resistance which appeared to be normal. These were generally patients with mild hypertension (60, 61) In the late 1950-ties and early 1960-ties a few papers describing a high cardiac output in presumably early hypertension were published (11 16, 29) The concept was then formed that in several types of hypertension, including essential hypertension in man, an increased cardiac output might be the first hemodynamic disorder The raised blood pressure would then start a series of reactions in the resistance vessels and in the heart, thus gradually changing the hemodynamic pattern towards a circulatory system characterized by an increased total peripheral resistance and a subnormal or low cardiac output (20) Thus the increased resistance might rather be a *secondary* phenomenon. The mechanism behind the primary increase in cardiac output was unknown.

Studies in animals

In the 1960-ties important observations on the hemodynamic alterations in the first phase of renal hypertension in rats were made by Ledingham and Cohen (36) They demonstrated that when the blood pressure started to rise, there was a transient increase in the cardiac output. The total peripheral resistance was initially normal, but then rose simultaneously with a drop in the cardiac output. The significance of these results was questioned because of animal variability and inaccuracies in the methods used for determination of the cardiac output in the rats. In similar experiments in dogs no initial increase in cardiac output was found (50)

However several years later a similar transient phase of increased cardiac output in the starting phase of hypertension in dogs with renal hypertension was reported from the Cleveland group by Ferrario et al. (17 18) In very elegant experiments flowmeters were implanted in trained dogs made hyper

leader His deeply felt responsibility as a doctor towards his patients as a teacher towards his students and pupils and as a servant of the state and a fellow citizen towards the society as a whole, has made a lasting impression upon those who have had the privilege of working together with him.

Although his scientific work, mostly in the field of cardiology has been of considerable importance, his main contribution to the field of clinical research has been that of the administrator, providing the tools for his coworkers He took the initiative to a most fruitful collaboration between Medical Department A and The Chr Michelsen Institute for Science and Intellectual Freedom in the late fifties His initiative led to pioneer work in the field of medical engineering an activity which has given Medical Department A a special project profile ever since The first Department of Clinical Physiology in the country was realized as a result of his initiative. He simultaneously allocated substantial resources for clinical research inside his own department, and successfully fought for the establishment of a further professorship to strengthen its research potential. On the nation scale he has been an outstanding member of the Norwegian Council of Cardiovascular Diseases and has served as its chairman from 1969 to 70 He has also been one of the Norwegian Redactores of Acta Medica Scandinavica for many years.

As a doctor he has practiced common sense with uncommon skill and with an unusual sense for what is to turn up He adopted the modern version of early mobilization after myocardial infarction about 25 years ago and can quote himself on anticoagulant therapy from the roaring fifties without feeling ashamed He was among the first to underline the social, psychological and ethical aspects of internal medicine and to stress the importance of health economy In these matters his voice has been heard in several books in an uncounted number of articles in newspapers and medical journals, at meetings for lay and learned in the radio and on television these activities have made him known all over the country His efforts have without doubt been of great importance for our national attitude towards doctors hospital and the health service in general.

He has been an unfatigable advocate for the importance of sport and out-door life for the national health standard, and is himself a formidable sportsman his activities including skiing tennis, touring mountain climbing and in his younger days even boxing He has served as a great example for his colleagues as well as for the lay man

Among his most prominent personal qualities are his vitality his attitude of deep responsibility and a remarkable combination of civil courage with an extraordinary sense of what is just and of fair play He enters persons and problems through the front door Apart from this he has that special quality which denies definition which may be observed in members of a family that has served the State for several generations Those who know him well know him as a man of good humour this a times formidable worrior for what he feels is just and important is a very kind man

When he now resigns, one of this century's great personalities in Norwegian medicine will be out of office We all — his coworkers and pupils — give him our most heartfelt thanks for the years passed and our best wishes for many active years to come

Jarle Ofstad

Acta Med Scand Suppl. 603 1—14 1977

Hemodynamic alterations in hypertension — spontaneous changes and effects of drug therapy A review

Per Lund-Johansen

Pioneer studies in man

The hemodynamic mechanisms responsible for the increased blood pressure in the various types of hypertension in animals and man have now been studied for almost 30 years. In the pioneer studies in man — using right heart catheterization — usually patients with rather severe hypertension were studied. In most patients it was found that the major disturbance in the central hemodynamics was an increased total peripheral resistance and the cardiac output during rest situation was normal or only slightly reduced as long as heart failure was not present (4 59 60 61)

These findings led to the conclusion that the *cardinal* and *primary* hemodynamic disorder in most types of hypertension was an increased total peripheral resistance based on functional and/or structural changes in the resistance vessels

However a few patients in the older studies had a high cardiac output and a total peripheral resistance which appeared to be normal. These were generally patients with mild hypertension (60, 61) In the late 1950-ties and early 1960-ties a few papers describing a high cardiac output in presumably early hypertension were published (11 16 29) The concept was then formed that in several types of hypertension including essential hypertension in man, an increased cardiac output might be the first hemodynamic disorder The raised blood pressure would then start a series of reactions in the resistance vessels and in the heart, thus gradually changing the hemodynamic pattern towards a circulatory system characterized by an increased total peripheral resistance and a subnormal or low cardiac output (20) Thus the increased resistance might rather be a *secondary* phenomenon. The mechanism behind the primary increase in cardiac output was unknown.

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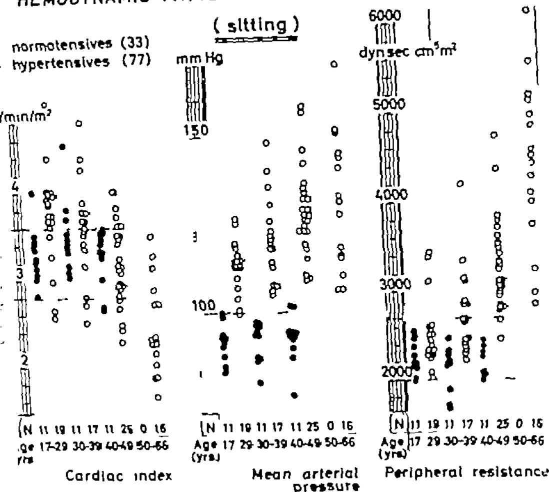


Fig 1 Hemodynamic pattern at rest in 77 hypertensive and 33 normotensive males.

udy was confined to men 93 hypertensives nd 48 normotensives (41) The main part f the work was devoted to the central hemodynamics at rest and during exercise The majority of the patients participating in hese experiments belonged to the WHO s group I and were less than 50 years of age

The results from this study clearly demonstrated that the hemodynamic mechanisms responsible for the increased blood pressure differed for the various age groups. During rest situation (sitting) the calculated *total peripheral resistance* fell within the „normal" range in all but 3 of the 19 patients below 30 years, and in 10 of the 17 patients 30–39 years old. It increased however in all but 2 of the 25 patients 40–49 years old (Fig 1) The mechanism behind the increased blood pressure in those having an apparently normal total peripheral resistance was an increased cardiac output. It could of course be argued that although the calculated total peripheral resistance fell within the "normal" range, it was still inappropriately increased *If* the function of the resistance vessels had been entirely normal, one should have expected vasodilation as a response to the increased cardiac *output*, thus keeping the

tensive by wrapping one kidney in cellophane (17) and later by inducing renal artery stenosis by an externally adjustable clamp on one renal artery (18) In both these models there was a phase with a significantly elevated cardiac output, the rise in resistance lagging behind

In recent years this has also been established in animals made very acutely hypertensive by stimulation of the stellate ganglion that in the starting phase the cardiac output is increased. Within a period of only 7 days there is a fall in the cardiac output and a 35% increase in the resistance. This first increase in the resistance is probably mediated by the sympathetic nervous system since it can be prevented by phenoxybenzamine (38)

Thus in all these *acute* experiments the initial increase in the cardiac output seems to play an important role in the starting phase of hypertension However there is a large gap between these animal models and the most common type of hypertension in man. Thus considerable interest has been focused on the hemodynamics of spontaneously hypertensive rats (SHR) the animal model showing many similarities with essential hypertension in man. Studies of this animal by Pfeffer & Frohlich (51) have shown that when the rats are young (< 12 weeks) and in a pre hypertensive phase they usually have a high cardiac output a high heart rate and a normal total peripheral resistance. With advancing age the pattern changes.

Other important observations from recent studies of animal hypertension should be emphasized. It is surprising how *rapidly* biochemical and morphological changes appear in the myocardium and in the resistance vessels when the blood pressure is increased In renal hypertension biochemical changes may be found in the myocardium and in the resistance vessels already a few days after the pressure is raised indicating increased growth of muscle and connective tissue in these structures (53) In the SHR,) the heart (measured b) *thickness/ ratio in left ventricle) is seen at 4 mo* age (15)

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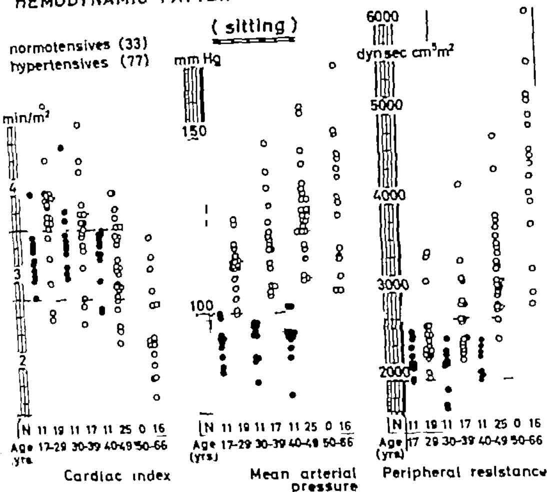


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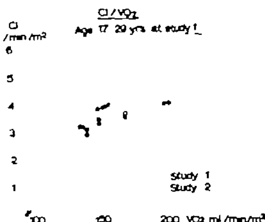


Fig 3. Cardiac index (CI) and oxygen consumption (VO_2) at rest sitting in young hypertensives. Study 1 in 1965–66, study 2 10 years later

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In subjects in their 40-ties or older the total peripheral resistance was increased at rest as well as during exercise. The stroke volume was subnormal not only during exercise but also during rest situation (sitting). In a small group of subjects with severe hypertension in WHO group II or III very marked elevation in peripheral resistance and reduction in cardiac output was found.

In contrast, in the 33 *normotensive* control subjects there were no significant differences between the hemodynamic patterns at rest or during exercise over the three decades (from 20 to 49 years).

Our most important findings have been confirmed by others (1 56) and the results have also been discussed in several review articles (2, 42 44 57). A further discussion of the findings will thus not be repeated here.

The *mechanism* behind the high cardiac output and the high heart rate in the young hypertensive group is not understood. An overactivity of the sympathetic nervous system is frequently brought into the discussion, but the role of the sympathetic nervous system in the early phase of essential hypertension is still uncertain (40). An imbalance between the sympathetic and the parasympathetic nervous system has been discussed (53). It should also be emphasized that this study (41) demonstrated that already in subjects in *their* 20-ties with mild essential hypertension and in whom no complications could be detected by clinical methods, the exercise test revealed that the function of the resistance vessels during exercise was abnormal and *that* the stroke volume during exercise was subnormal. The possible mechanisms behind these changes will be discussed later.

The results from this cross-sectional study demonstrated the following hemodynamic pattern in essential hypertension. A high flow/ normal" resistance pattern dominating in the youngest group and a low flow/high resistance pattern in the oldest. If these subjects were characteristic for a population of patients with essential hypertension, one should expect that the cardiac index would decrease and that the resistance would increase in the *majority* of the patients over the years — in agreement with Folkow's concept of the restructuring of the high pressure compartments in hypertension (20).

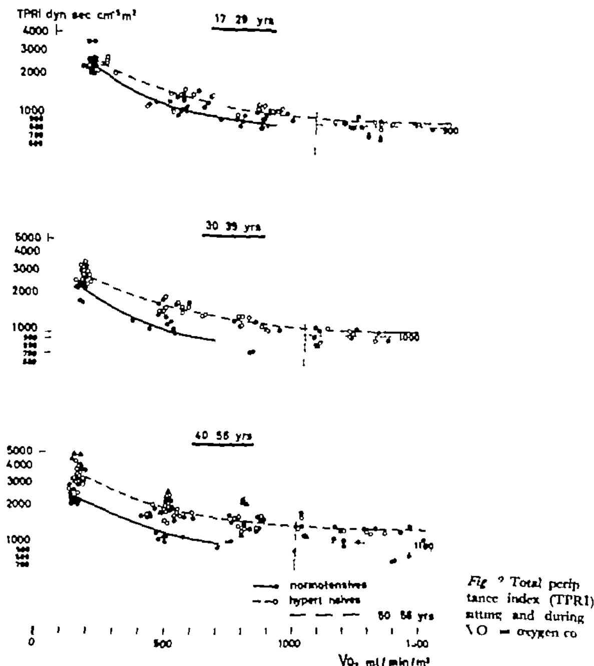


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blood pressure normal These considerations become more clear when the patients were studied during muscular exercise. Then it was obvious that the function of the re sistance vessels was abnormal in *all* age groups including the youngest (Fig 2) The resistance did not fall to the same low levels as in the normotensive controls It was therefore concluded that the calculated total peripheral resistance was abnormal in subjects with essential hypertension already in their 20-ties.

In the youngest group the resting *cardiac index* was significantly higher than in normotensive age matched controls and h in hypertensives one or two decades Thus a high cardiac pump function characteristic feature in the early sive phase The high cardiac index rest was caused by an increased hear the stroke index being normal

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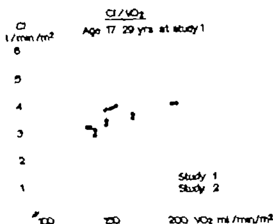


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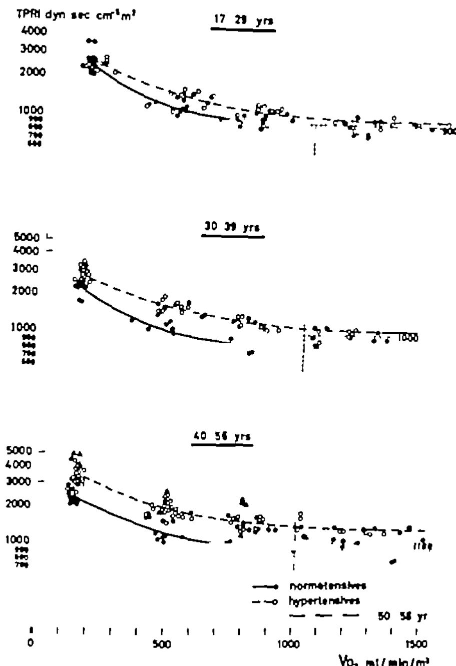


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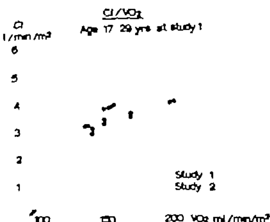


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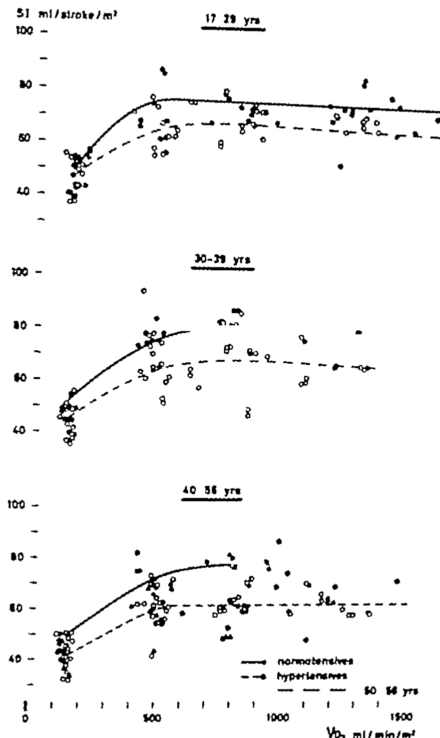


Fig 4 Stroke index (SI) sitting and during exer

Only prospective studies can tell if these assumptions are correct

Longitudinal studies So far no real systematic long term studies on the central hemodynamics in subjects with untreated essential hypertension have been made that include both rest and exercise situations In one study from the U S A by Eich et al (12) the interval between the two examinations was only about 4 years and the co prevalent during study 1 and study 2 different. In another study from Swed Eliasch et al (14) the first study was using heart catheterization and the using a dye dilution technique In a study from Holland by Birkenhäger et al the patients had been treated and taken off drugs shortly before the study

10 year follow-up of central hemodynamics in untreated patients

At least 10 years have now elapsed since the 77 hypertensive patients in the 1967 study were first examined. They have now all been restudied clinically and the untreated subjects in the two youngest age groups have also been examined hemodynamically

Clinical data. The oldest age group (50—66 years in 1967 n=16) included 11 patients in WHO stage II or III the mean blood pressure for the whole group being 184/104 mmHg Information has been available for all patients. They all received drug treatment mainly diuretics. Twelve were dead. Only 4 were alive, and these were still able to work at the ages of 62 64 64 and 72 years. All the living patients were initially in WHO stage I or II Age group III (40—49 years old) included 25 patients, all but two in WHO stage I The mean blood pressure at the first study was 169/102 mmHg One subject died of myocardial infarction after 5 years on diuretic therapy the rest were alive. Only 5 patients in this group had not been on drug therapy (They have now been restudied hemodynamically but due to the small number they will not be included in this presentation.)

Age group II (30—39 years old) all in WHO stage I had a mean blood pressure of 160/99 mmHg when first studied Information was available in all patients. One subject died at the age of 44 years of heart and lung insufficiency The remaining 16 patients were all alive all still in WHO group I Thirteen patients had been untreated and they have all been restudied. In the youngest age group (17—29 years old) all in WHO stage I the mean blood pressure was 150/92 mmHg Two subjects were out of the country Of the remaining 17 all were alive apparently healthy and still in WHO stage I Sixteen have been untreated and 15 have been restudied.

At the clinical follow-up study all untreated subjects still had hypertension or borderline hypertension. None had become normotensive over the 10 years None had developed any complications detectable by clinical examination, ophtalmoscopy ECG urine or blood analysis.

Hemodynamic restudy Exactly the same methods were used in study 2 as in study 1 On both occasions the subjects were studied as outpatients in the morning 2 hours after a light breakfast. The subjects were studied at rest sitting and during muscular exercise at 50 100 and 150 watt. Oxygen consumption was measured by Douglas bag and Scholander technique, heart rate by EGG cardiac output by due dilution method, and the blood pressure recorded intraarterially and followed continuously at rest and during exercise.

Details about the restudy will be published elsewhere (48 49) and thus only the major findings will be presented here (Figs 5—8)

The oxygen consumption which was significantly above normal in the youngest age group 10 years ago had decreased significantly to values close to those found in the age group one decade older 10 years ago During exercise, there were no consistent changes in the oxygen consumption.

The blood pressure Somewhat surprisingly during rest situation there were no consistent changes in systolic, diastolic or mean arterial blood pressures, either in group I or group II. At the highest work load, however the diastolic and mean arterial pressure had increased significantly in both age groups.

Cardiac index Both in age group I and age group II the cardiac index during rest had decreased significantly The changes were very uniform. The cardiac index had decreased in all but two subjects in age group I and in all subjects in group II During exercise the cardiac index had decreased significantly in both age groups.

Heart rate and stroke index. The results

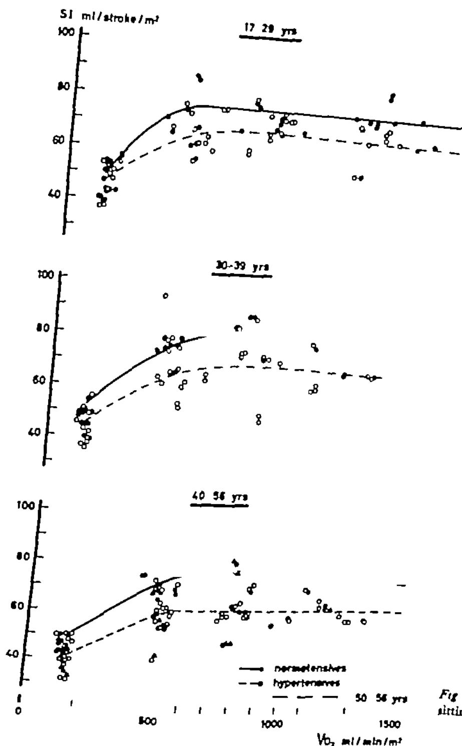


Fig 4 Stroke index (SI) at sitting and during exercise.

Only prospective studies can tell if these assumptions are correct.

Longitudinal studies So far no real systematic long-term studies on the central hemodynamics in subjects with untreated essential hypertension have been made that include both rest and exercise situations In one study from the U S A. by Eich et al (12) the interval between the two examinations was only about 4 years and the conditio prevalent during study 1 and study 2 different. In another study from Sweden Eliasch et al. (14) the first study was using heart catheterization and the secon using a dye dilution technique In a study from Holland by Birkenhager et al (f the patients had been treated and taken off drugs shortly before the secon study

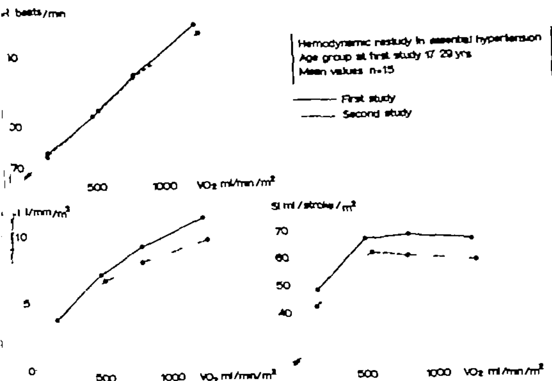


Fig. 7 Heart rate, cardiac index and stroke index at rest sitting and during exercise in youngest age group in studies 1 and 2.

These changes could be due to the process of aging or aging plus increased blood pressure. As the normotensive control group has not been restudied, the changes by normal aging have to be elucidated by results from the 1967-study (41) and from results by other investigators who studied the evolution of the circulatory changes in various age groups (5 10 27 31) These studies have shown that for patients with normal blood pressure no significant changes occur in the cardiac index either at rest or during exercise by aging from the 20-ties to the 40-ties. As reported, the evolution has been quite different in the hypertensives and thus it is reasonable to assume that the decrease in flow and increase in resistance must be a consequence of the high blood pressure. Similar trends were also found in the follow up studies by Eich et al. (11) Ellasch et al. (14) and Birkenhäger et al (8)

Discussion

What are the deeper mechanisms behind these hemodynamic changes observed in animal and human hypertension? First of all it must be emphasized that the mechanism initiating the acute rise in blood pressure in the various animal models described, probably are very different from the starting phase of essential hypertension in man. Thus in the renal model the elevated cardiac output is caused by an increase in both the stroke volume and the heart rate while in man with essential hypertension the heart rate is increased. No satisfactory explanation of the increased heart rate and cardiac output has been given, and somewhat disappointingly all these hemodynamic studies have brought us no closer to the etiology of essential hypertension. We still do not know whether it is a separate disease or just a quantitative abnormality (52)

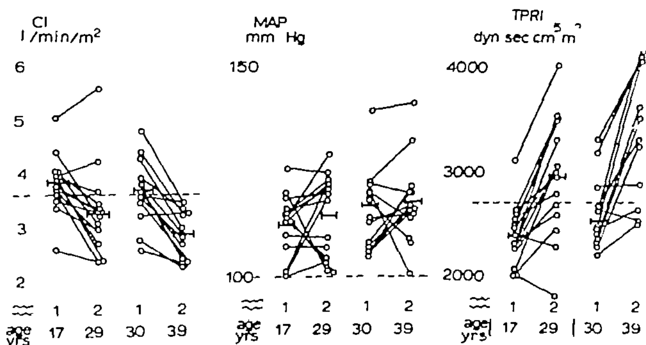


Fig 5. Cardiac index (CI) mean arterial pressure (MAP) and total peripheral resistance index (TRPI) rest sitting Study I in 1965–66 study 2 10 years later

showed that the most important cause of the reduction in the cardiac output was that the stroke index had decreased significantly at rest and during exercise (in both age groups)

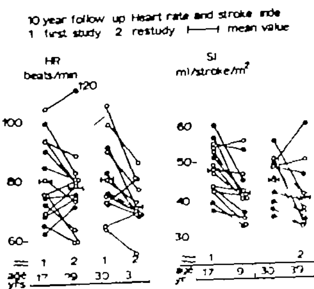


Fig 6. Heart rate (HR) and stroke index (SI) at rest sitting in studies 1 and 2

The heart rate did not show any ... changes either at rest or during exercise ... age group I but in age group II there w... significant decrease in the heart rate at ... but not during exercise.

Total peripheral resistance index The ... peripheral resistance index had increas... significantly in both age groups at rest ... well as during exercise The results were ... consistant and at rest the resistance h... increased in all but two subjects in the youn... est group and in all subjects in age grou... 30–39 years.

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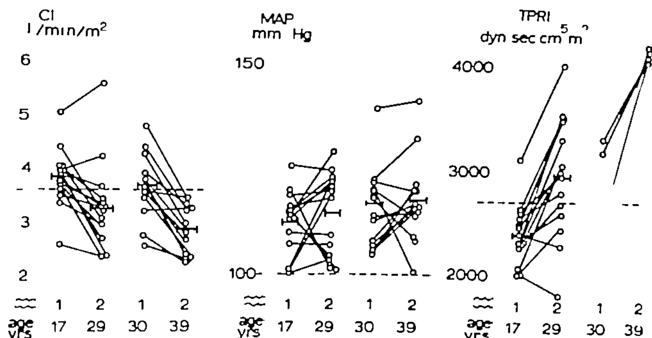


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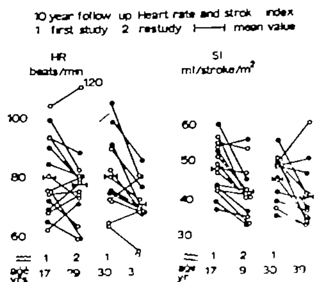


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HR beats/min
150
100
70
500 1000 VO_2 ml/min/m²

Hemodynamic restudy in essential hypertension
Age group at first study 17 29 yrs
Mean values n 15

—— First study
---- Second study

CI l/min/m²
10
5
0 500 1000 VO_2 ml/min/m²

SI ml/stroke/m²
70
60
50
40
500 1000 VO_2 ml/min/m²

Fig 7 Heart rate cardiac index and stroke index at rest sitting and during exercise in youngest age group in studies 1 and 2

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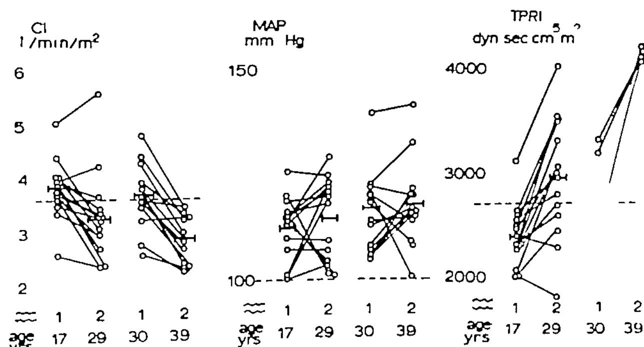


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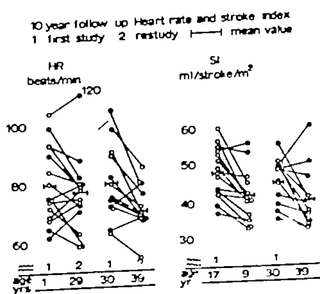


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It is beyond the scope of this article to discuss these important problems in detail but few recent studies will be reviewed briefly

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In mice made hypertensive by social -ress in special cages, blood pressure will eturn to normal if the mice are removed om the stress c ge with n a few days (30) I the sta is prolonged to 9 months (equivalent to 20–30 years in human life span) the hypertension becomes permanent and he left ventricular hypertrophy is irreversible. Recent studies seem to indicate that his is partly due to increasing formation of collagen and other connective tissue (22)

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Some studies in man also seem to indicate hat really long standing hypertension will) cause permanent changes in the resistance vessels In a recent study of muscle blood flow and peripheral resistance in persons who had been successfully operated for coarctation of the aorta, the calculated resistance in the arms was abnormal even several years after the operation (55)

In our laboratory we have studied the hemodynamic alterations induced by "long term" (1 year) therapy of the most commonly used antihypertensive drugs in established essential hypertension. The results have shown that the various antihypertensive drugs affect the hemodynamic abnormalities differently

The thiazide-diuretics initially reduce blood pressure by decreasing plasma volume, stroke volume and cardiac output, but after months of therapy the cardiac output returns to the pretreatment level and the peripheral resistance falls. The heart rate is not influenced either at rest or during exercise. Thus one of the important hemodynamic abnormalities is at least partially corrected, at rest as well as during exercise (43) The reduced stroke volume does not increase, however

The centrally acting sympathetic inhibitors like alphamethyldopa and clonidine have their most pronounced effect during the rest situation. The heart rate and the cardiac output are reduced and total peripheral resistance not much influenced. During exercise these drugs had relatively little effect on the blood pressure, and thus they don t seem to correct the hemodynamic abnormalities in such stress situations (46) The frequent side-effects make them less suitable in subjects who are without complaints prior to therapy

The beta-blockers induce an overcorrection of the increased heart rate resulting in marked bradycardia at rest and during exercise (47) On some beta-blockers there is a compensatory increase in the stroke volume, but never great enough to compensate completely for the reduction in the heart rate. The consequence is that the cardiac output is reduced at rest as well as during exercise. The arteriovenous oxygen difference is further increased. The total

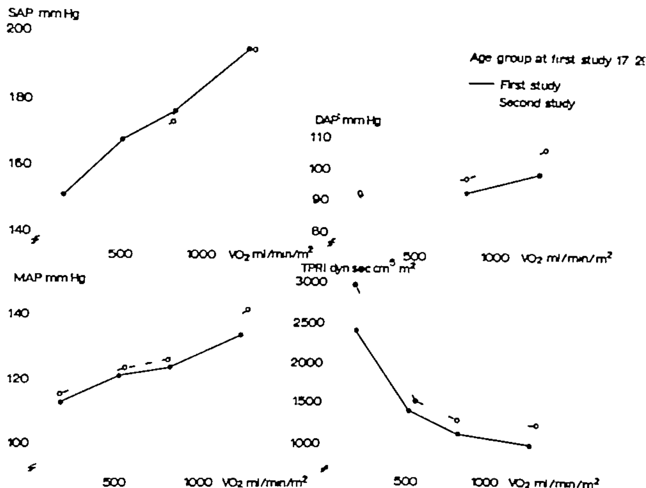


Fig 8 Systolic (SAP) diastolic (DAP) and mean (MAP) arterial pressure and total peripheral resist index (TPRI) at rest sitting and during exercise in youngest age group in studies 1 and 2

However it is possible that the observation of the central hemodynamics in presumably early essential hypertension have been more important in demonstrating how early the function of the resistance vessels and the pump function of the heart become disturbed It is interesting to compare the early alterations in the central hemodynamics in essential hypertension with the functional and structural changes in the SHR It has been shown recently that in SHR the cardiac output is high in the early phase (51) Soon however there is an increase in the wall thickness of the left ventricle and an increase in the wall thickness — left ventricular cavity ratio In rats with renal hypertension it has been shown that the compliance of the left ventricle soon becomes reduced This is demonstrated by infusion experiments When hypertensive and normal rats are infused with blood, the increase in left v tricular end diastolic pressure (LVFDP) sults in less increase in stroke volume in hypertensive rats than in the normotens (3 58) It is of course not possible to te these hemodynamic changes in man are to functional or structural changes H ever the results would seem to fit well v Folkows theories about the structural ch ges appearing in the heart and resista vessels when the pressure increases (

To learn more of the mechanisms in starting phase of essential hypertension pr ably even younger subjects must be stud Today there has been an increasing inte in the blood pressure in children and rec data suggest that essential hypertens starts much earlier than the age 1 20 (It is possible that hemodynamic studies an even earlier stage could reveal a pur

ncreased heart rate and cardiac output — without any disturbances in the total peripheral resistance or in the stroke volume during exercise.

Reversal of early changes in hemodynamics or in structure.

Is it possible to stop or to reverse the vicious circle operating in hypertension by removing the cause or by drug treatment? At what stage are the changes reversible?

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The peripheral *vasodilator prazosin* significantly reduces total peripheral resistance at rest and during exercise (45) There is an increase in the stroke volume during exercise and as a consequence an increase in the cardiac output. Since prazosin is not a pure vasodilator but also has an effect which prevents the marked tachycardia seen for other vasodilators, there are no significant changes in the heart rate Thus prazosin changes several of the hemodynamic disturbances in essential hypertension in the direction of normal However when given alone it is relatively ineffective in more severe hypertension.

The consequences of these different types of hemodynamic changes will have to be followed over several years A 3-year follow up study in a group of patients treated with a beta blocker demonstrated that the reduction in the heart rate still persisted and that the stroke volume was the same at the re study after 3 years as after 1 year Thus at least there had been no further deterioration in the heart pump function In a study of the peripheral circulation in patients with essential hypertension treated by drugs for 5 years signs of incomplete reversibility of the changes in peripheral resistance were found (28)

Thus at present it is not possible to answer the key question completely It is possible to correct some of the hemodynamic abnormalities using available drugs, but far more studies are necessary to find at what stage treatment with antihypertensive drugs should be started If one thinks of starting therapy in the hypertensive phase in man, then the d drug would of course have to be entir of sideeffects

Conclusion

Studies in animals and man have support to Folkow's theory about structuring of the cardiovascular syste hypertension Ten year follow-up h dynamic studies in untreated subjects mild essential hypertension in stage I have shown that the function o cardiovascular system has deteriorated than what should be expected from alone.

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The consequences of these different types of hemodynamic changes will have to be followed over several years A 3-year follow up study in a group of patients treated with a beta blocker demonstrated that the reduc tion in the heart rate still persisted and that the stroke volume was the same at the re-study after 3 years as after 1 year Thus at least there had been no further deteriora tion in the heart pump function In a study of the peripheral circulation in patients with essential hypertension treated by drugs for 5 years, signs of incomplete reversibility of the changes in peripheral resistance were found (28)

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Conclusion

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Comparison of analog and numerical computation of cardiac output from dye dilution curves

L. Markrid and P Lund-Johansen

dye dilution technique has become a established method of measuring car output (Q) both in clinical (1) and rimental work (2,3) In its standard a known amount m, of indicator is dly injected at one site in the circulation m and variation of blood dye concen on over time, c(t) monitored at a more l site. If the dye is uniformly mixed s the flow stream between the two sites, cardiac output may be calculated accord- to Stewart–Hamilton's equation (4)

$$Q = m / \int_0^\infty c(t)dt \qquad (E. 1)$$

denominator is the area under the centration-time curve after a correction been made for recirculated dye (Fig 1) is usually assumed that the descending t of the dye concentration curve in ence of recirculation follows an exponen l decay (5)

$$c(t) = c_a exp \{-\alpha(t\ t_a)\} \quad t > t_a \qquad (E.2)$$

ere t_a is any time point after the start nt t_a of the exponential fall The constants and t_a are usually found by determining straight line portion of $\ln c(t)$ or equi ently c(t) plotted against time on semi arithmic paper The area under the ponential part of the curve from the point t_a n then be found by integration

$$\int_{t_a}^{\infty} c_a exp \{-\alpha(t-t_a)\} \quad dt = c_a/\alpha \qquad (E. 3)$$

The calculation of the total area under the corrected concentration curve can be per formed manually (6) on digital computers (7) or by means of analog computation (8 9) Modifications of these three methods have been tested in our laboratory in connec tion with out-patient hemodynamic studies and will be described below

MATERIAL AND METHODS

182 dye curves from 20 male hypertensives were investigated, in 7 individuals before, and in 13 after α-methyldopa therapy (10) Indocyanine green, 5 mg in 2 ml was injected by the slug method through 60 cm long polythylene catheter introduced into an antecubital vein and advanced to the region of the left axillary vein or superior vena cava. The arterial dye concentration in blood withdrawn through a polyethylene catheter inserted into the left brachial artery was monitored via monochromatic cuvette densitometer (Chr Michelsens Institute, Dept. of Technology and Science, Bergen Norway) The densitometer signal was connected to a special purpose built analog computer (developed by the same institute) for integration of the dye concentration curves. In addition the curves were recorded on photographic paper by means of a UV-recorder. The handling of the dye solution, the technique for injection and flushing, description of the apparatus and calibration procedures have been reported earlier (11).

The studies were performed with the patients at rest, both in the supine and in the sitting position, as well as sitting on an ergometer bicycle during graded muscular exercises (50, 100, and 150 W). A wide range of cardiac outputs (4.5–20 l/min) was thus obtained.

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Acta Med Scand Suppl. 603:15–21 1977

Comparison of analog and numerical computation of cardiac output from dye dilution curves

L. Markrid and P. Lund-Johansen

…e dye dilution technique has become a …ll established method of measuring car…ac output (Q) both in clinical (1) and …perimental work (2,3). In its standard …m a known amount m, of indicator is …idly injected at one site in the circulation …tem and variation of blood dye concen…tion over time, c(t), monitored at a more …tal site. If the dye is uniformly mixed …om the flow stream between the two sites, … cardiac output may be calculated accord… to Stewart–Hamilton's equation (4)

$$Q = m / \int_0^\infty c(t)dt \qquad \text{(E. 1)}$$

…e denominator is the area under the …centration time curve after a correction … been made for recirculated dye (Fig. 1). …t is usually assumed that the descending …t of the dye concentration curve in …sence of recirculation follows an exponen…l decay (5)

$$c(t) = c_a \exp\{-\kappa(t - t_a)\} \quad t > t_s \qquad \text{(E.2)}$$

…ere t_a is any time point after the start …nt t_s of the exponential fall. The constants … and t_s are usually found by determining … straight line portion of $\ln c(t)$ or equi…ently c(t) plotted against time on semi…arithmic paper. The area under the …ponential part of the curve from the point t_a …n then be found by integration

$$\int_{t_a}^\infty c_a \exp\{-\kappa(t - t_a)\}\, dt = c_a/\kappa \qquad \text{(E. 3)}$$

The calculation of the total area under the corrected concentration curve can be performed manually (6), on digital computers (7), or by means of analog computation (8, 9). Modifications of these three methods have been tested in our laboratory in connection with out-patient hemodynamic studies and will be described below.

MATERIAL AND METHODS

182 dye curves from 20 male hypertensives were investigated, in 7 individuals before and in 13 after α-methyldopa therapy (10). Indocyanine green, 5 mg in 2 ml was injected by the slug method through a 60 cm long polythylene catheter introduced into an antecubital vein and advanced to the region of the left axillary vein or superior vena cava. The arterial dye concentration in blood withdrawn through a polyethylene catheter inserted into the left brachial artery was monitored via a monochromatic cuvette densitometer (Chr. Michelsens Institute, Dept. of Technology and Science, Bergen, Norway). The densitometer signal was connected to a special purpose built analog computer (developed by the same Institute) for integration of the dye concentration curves. In addition the curves were recorded on photographic paper by means of a UV-recorder. The handling of the dye solution, the technique for injection and flushing, description of the apparatus and calibration procedures have been reported earlier (11).

The studies were performed with the patients at rest, both in the supine and in the sitting position, as well as sitting on an ergometer bicycle during graded muscular exercises (50, 100, and 150 W). A wide range of cardiac outputs (4.5–20 l/min) was thus obtained.

Integration of dye concentration curves

1 *Manual method.* The baseline value recorded just before the initial appearance of dye was chosen as the zero reference, and the arterial dye concentration ordinates were read from the photographic recordings at one second intervals. The descending slope of the curve was plotted on semilogarithmic paper the best visual fit to a straight line was drawn, and the curve was extrapolated down to about 4% of its peak value. The area was then calculated according to the rectangular rule by summation of the ordinate values (12) The whole procedure was always carried out by the same technician.

2 *Digital method* The same set of concentration coordinates used in the manual computation were also read into a Hewlett Packard 9820 A desk computer A program was written which calculated the area from $t = 0$ to $t = t_n$ using Simpson s rule (12) and the remaining area according to Eqn. (E.3) Simpson s rule was modified by incorporation of an additional term in the case of an even number of ordinates (Appendix) The constant κ in Eqn. (E. 3) was determined by the method of least squares (13) from the following linear first order regression model

$$y_i = \ln c_i = \lambda - \kappa t_i + \varepsilon_i \quad i = 1,2 \quad n \quad (E. 4)$$

The ordinates were chosen in the interval from the first encountered below $0.7 \cdot c_m$ to the first below $0.3 \cdot c_m$, where c_m was the maximum concentration determined (Fig 1) This wide range was necessary in order to obtain more than one regression point with one-second intervals in the group with highest cardiac output. Careful examination of the curves showed that the effect of recirculation in this range of ordinates was negligible with present patient material. The computer was also programmed to compute mean transit times and central vascular volumes (4) on the basis of dye appearance

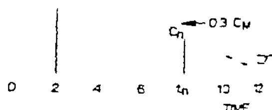


Fig 1 Dye dilution curve with one-second i[…] samples, prepared for numerical integrat[…] C_m = maximal dye concentration read, C_n ordinate being used in Simpson s rule. For [...] explanation, see text.

times, O_2 consumption obtained from r[…] ratory gas analysis, and a series of [...] hemodynamic parameters.

3 *Analog method.* The analog com[…] utilized the following formula (14) fo[…] area estimation (referring to Fig 2)

$$A = \int_0 c(t)dt - \int^b c(t)dt \ (k-1) \quad (I$$

The integral limits a and b can as[…] two different values, depending on the in[…] ment setting (setting I was used in this s[…]

$$c(a) = c_p \begin{cases} 0.6 & \text{setting I} \\ 0.7 & \text{» II} \end{cases}$$

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$$k = c(a)/c(b) = 1.5 \quad (I$$

The instrument was supplied with a hold circuit to assure correct

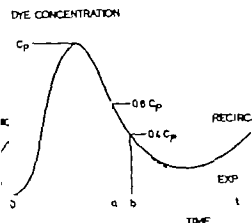


Fig. 2. Illustration of parameters used in analog computation of dye dilution curve areas. C_p = peak dye concentration, a, b = integral limits, see text.

memory for the calibration deflection. It has been described in detail elsewhere (15)

Statistical analysis

The digitally computed cardiac output was considered to be the most reliable one (2,6) and was consequently used as the reference, X. The mean relative deviation $\bar{e}$, between X and the value Y obtained by either of the two other integration methods, was calculated according to the formula

$$\bar{e} = (1/n) \sum_{i=1} e_i = (1/n) \sum_{i=1} (Y_i/X_i - 1) \quad n = 182 \qquad (E. 8)$$

The coefficient of relative variation s, was obtained from

$$s^2 = \sum_{i=1} (e_i - \bar{e})^2 / (n - 1) \qquad (E. 9)$$

To illustrate the functional dependence between Y and X, the following first and second order linear regression models were used

$$Y = a + bX_i + \epsilon_i \quad i = 1,2 \ldots n \qquad (E.10)$$

$$Y = a' + b' X_i + c' X_i^2 + \epsilon_i \qquad (E. 11)$$

The parameters (polynomial coefficients) were estimated by the method of least

MANUAL CALCULATION (Y)

Y = 1.020X

n = 182

DIGITAL COMPUTER (X)

20 15 10 5

5 10 15 20

Fig. 3. Relationship between manually calculated and digitally computed values of cardiac output (l/min). Open circles represent two or more approximately coincident points. The first order regression line determined by the method of least squares is drawn.

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$$y_i = \ln c_i = \lambda - \varkappa t_i + e_i \quad i = 1, 2, \ldots n \quad (E\ 4)$$

The ordinates were chosen in the interval from the first encountered below $0.7 \cdot c_m$ to the first below $0.3 \cdot c_m$, where c_m was the maximum concentration determined (Fig 1). This wide range was necessary in order to obtain more than one regression point with one-second intervals in the group with highest cardiac output. Careful examination of the curves showed that the effect of recirculation in this range of ordinates was negligible with present patient material. The computer was also programmed to compute mean transit times and central vascular volumes (4) on the basis of dye appearance

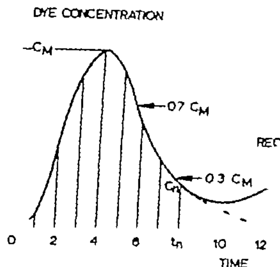


Fig 1 Dye dilution curve with one-second samples, prepared for numerical integratic C_m = maximal dye concentration read, C_n ordinate being used in Simpson's rule. For explanation, see text.

times O_2 consumption obtained from ratury gas analysis, and a series of hemodynamic parameters

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$$A = \int_0^a c(t)dt + \int^b c(t)dt/(k-1) \quad ($$

The integral limits a and b can two different values, depending on the ment setting (setting I was used in this st

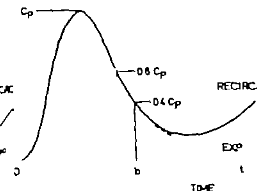
$$c(a) = c_p \begin{cases} 0.6 & \text{setting I} \\ 0.7 & \text{» II} \end{cases}$$

$$c(b) = c_p \begin{cases} 0.4 & \text{setting I} \\ 0.47 & \text{» II} \end{cases} \quad ($$

where c_p is the peak concentrati n me To give correct total area the con tant k be chosen

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$$Y = a + b X_i + c X_i^2 + \varepsilon_i \qquad (E.\ 11)$$

The parameters (polynomial coefficients) were estimated by the method of least

MANUAL CALCULATION (Y)
Y 1.020X
n 182
DIGITAL COMPUTER (X)

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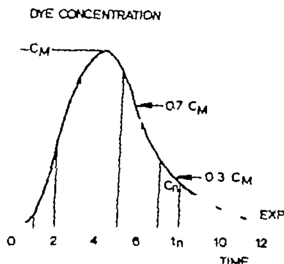


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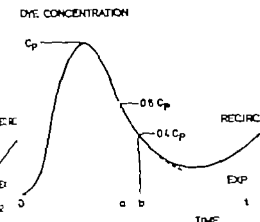


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The parameters (polynomial coefficients) were estimated by the method of least

20
MANUAL CALCULATION (Y)
Y 1.020X
182
15
10
5
DIGITAL COMPUTER (X)
5 10 15 20

Fig. 3. Relationship between manually calculated and digitally computed values of cardiac output (l/min). Open circles represent two or more approximately coincident points. The first order regression line determined by the method of least squares is drawn.

squares (13) The coefficient values thus obtained are given with 95% confidence limits With the second order model precautions were taken in the digital data analysis to minimize the effect of round-off errors (16) To test which of the coefficients differed significantly from zero a two-sided t test was performed (13)

Results

The cardiac output values determined from all the 182 dye dilution curves by the three different integration methods are shown in Figs. 3 and 4 A straight line relationship was found between the manually computed and the digitally computed values on the basis of visual inspection The mean relative deviation e, was +0 020 and the coefficient of relative variation was 0 010 The model in Eqn (E. 10) was adopted and the subsequent regression analysis gave the following estimates â = 0 008 ± 0 018 l/min (not significantly different from zero, P > and $\hat{b}$ = 1 020 ± 0 0040 (highly sign difference from both zero t value = and from 1 t value = 9 62) The corr coefficient was r = 0.9996 and the esti standard error of the Y_i's was s_y = l/min.

The mean relative deviation ē betwe analog and the digital computation +0 0068 and the coefficient of rel variation s = 0 065 Fig 4 which depic relationship between the two methods, that a first order regression model longer appropriate. Consequently the se order model given by Eqn (E. 11) tried with the resulting estimates â = −1 ± 1 006 l/min $\hat{b}$ = 1 4085 ± 0.0003, c = −0 02125 ± 0 0005 min/l All coefficients were significantly different zero (P < 0 005) The coefficient of mul determination was r^2 = 0 974 and the es ated standard errors of the Y_i's was 0 67 l/min

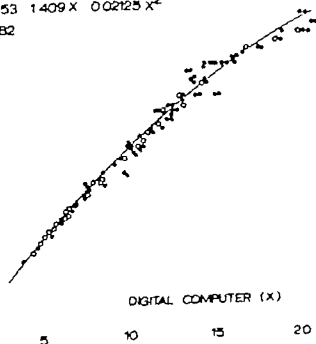


Fig 4 Graph of analog versus digi computed values of cardiac ou (l/min) Open circles represent tw more approximately coincident po The second order regression curve de mined by the method of least squ is drawn.

Discussion

In a previous study it has been shown that the dye method used in these experiments and the manual calculation of the curve area gave flow values within 1 % deviation from true values determined by flowmeter or direct measurements of flow by a measuring cylinder and a stop-watch (2)

The regression analysis showed that the manual calculation performed according to the rectangular rule as described above, overestimated the cardiac output systematically by 2% or equivalently underestimated the area of the dye dilution curve by approximately the same amount. This underestimation of curve area may partly be explained by the area lost when cutting off at approximately 4% of the peak value in the semilogarithmic plot in the manual calculation (17). The small coefficient of relative variation must be viewed in light of the fact that the two methods use the same set of input data. Small inaccuracies in reading the concentration ordinates will therefore affect the two methods in the same direction.

Both the plot of the analog computed cardiac outputs against those obtained in the digital computation (Fig 4) and the regression analysis over the total range of values, revealed a relationship between the two methods that deviated from a straight line through origo The coefficient of variation was also markedly greater than that between the two numerical integration methods. This does not automatically dismiss the analog computation method, however The errors contributing to this discrepancy must first be studied thoroughly Some of these errors will be briefly mentioned below 1) Different baseline drifts in the analog integrator and the UV-recorder will result in different curve areas. 2) A drifting zero in the photocell or the densitometer may cause an incorrect zero reference for the analog integrator since an ample time margin must be allowed between releasing the zero hold button and the start point of the dye curve. An unsteady blood dye concentration resulting from a short time interval between two successive injections will cause the same trouble. A more correct start point level can be found in the numerical calculation. 3) There may also be some ambiguity in determining the concentration ordinates on the photographic paper from the UV-recorder especially in the presence of noise or when the trace is blurred. Examination of this error by reading the ordinates on the inner and outer contour of the curves gave a maximum difference in the respective areas of about 4%

This study includes many dye dilution curves representing very high cardiac outputs, altogether 43 out of 182 values were greater than 15 l/min, with consequently small curve areas. Small variations in the analog input signal or the amplification process may therefore cause large relative changes in the group with high cardiac outputs. This is illustrated by a greater scatter of points in the upper right corner of Fig 4 The deviation from a straight regression line, which is also most prominent in this region, is difficult to explain, but will be subjected to further studies in the near future. In the interval 5—15 l/min, the maximum deviation of the predicted regression curve $\hat{Y} = -1.53$ l/min + 1.409 X − 0.02125 min/l X^2 from the line of identity Y = X, is +0.41 l/min at X = 8.48 l/min. The differences at the lower and upper endpoints are −0.02 and −0.18 l/min respectively Exclusion of all points with X > 15 l/min and recalculation using a first order regression model gave the following estimates $\hat{a}$ = 0.157 ± 0.082 l/min (significantly different from zero, P < 0.001) and b = 1.006 ± 0.025 (not significantly different from 1 P > 0.6) The deviation from the line of identity is then maximal at the upper endpoint X = 15 l/min,

squares (13). The coefficient values thus obtained are given with 95% confidence limits. With the second order model, precautions were taken in the digital data analysis to minimize the effect of round-off errors (16). To test which of the coefficients differed significantly from zero a two-sided t test was performed (13).

Results

The cardiac output values determined from all the 182 dye dilution curves by the three different integration methods are shown in Figs. 3 and 4. A straight line relationship was found between the manually computed and the digitally computed values on the basis of visual inspection. The mean relative deviation e_r was +0.020, and the coefficient of relative variation was 0.010. The model in Eqn. (E. 10) was adopted and the subsequent regression analysis gave the following estimates: $\hat{a}$ = 0.008 ± 0.018 l/min (not significantly different from zero, P ... and $\hat{b}$ = 1.020 ± 0.0040 (highly si... difference from both zero: t value = ... and from 1: t value = 9.62). The corr... coefficient was r = 0.9996 and the esti... standard error of the Y_i's was s_{xy} = ... l/min.

The mean relative deviation ē betwe... analog and the digital computatio... +0.0063 and the coefficient of re... variation s = 0.065. Fig. 4 which depi... relationship between the two methods, that a first order regression model ... longer appropriate. Consequently the se... order model given by Eqn. (E. 11) ... tried with the resulting estimates: $\hat{a}$ = – ... ± 1.006 l/min, b = 1.4085 ± 0.0003 ... c = –0.02125 ± 0.0005 min/l. Al... coefficients were significantly different ... zero (P < 0.005). The coefficient of mu... determination was r^2 = 0.974 and the e... ated standard errors of the Y_i's was ... 0.67 l/min.

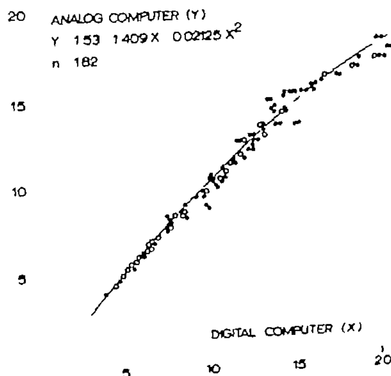


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where it amounts + 0.23 l/min. This indicates that the values predicted from the analog computed curves agree well with those from the numerical calculation in the interval of cardiac outputs found during rest and mild exercise. The correspondance between the two methods of integration is better than that reported by other authors (9).

In the clinical situation the analog computer here described should be considered a reliable instrument when dealing with normal dye dilution curves. It will give the cardiac output value within a minute of dye injection.

The digital computation takes longer time to complete, but has the advantages of giving better control when abnormal curve forms occur. It enhances the possibilities for providing diagnostic error notes and simultaneous calculation of other parameters like mean transit times, central vascular volumes, and curve variances (17). In our laboratory this method now is used routinely and has been incorporated into a more elaborate program system for the analysis of hemodynamic data from hypertensive patients. Compared with the manual method, it is obviously time saving.

Appendix

By Simpson's rule (12) the area under a curve passing through three points with equispaced abscissas is approximated by the area of a parabola passing through the same points. It is generalized to include an uneven number of points n by summing the contribution from a series of such triplet points. With abscissa distance h betwen the points the formula is

$$A = (h/3)\ (c_1 + 4c_2 + 2c_3 + 4c_4 + \dots + 4c_{n-1} + c_n) \qquad (A.\ 1)$$

where c_i, i = 1, 2, ..., n are the ordinate values.

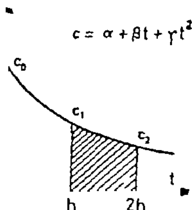


Fig. 5. Explanation of symbols used in the deriv... of a modified Simpson's rule.

When n is an even number the or... Simpson's formula may be used to calc... the area under the curve passing the... the first (n 1) points. The rest area und... parabola fit to the last three points limited by the verticals passing through last two points (cross-hatched area in Fi... can be found as follows.

Let the parabola be represented by

$$c(t) = \alpha + \beta t + \gamma t^2 \qquad (A$$

The rest area can be found by sim... integration

$$A_{rest} = \int_h^{2h} c(t)dt = h\ [\alpha + 3\beta h/2 + 7\gamma h \qquad (A$$

The relationship between the coeffici... (α, β, γ) and the ordinates (c_0, c_1, c_2) is m... readily expressed in matrix form

$$\begin{pmatrix} 1 & 0 & 0 \\ 1 & h & h^2 \\ 1 & 2h & 4h^2 \end{pmatrix} \begin{pmatrix} \alpha \\ \beta \\ \gamma \end{pmatrix} = \begin{pmatrix} c_0 \\ c_1 \\ c_2 \end{pmatrix} \qquad (A.$$

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$$\alpha = c_0$$
$$\beta = (-3c_0 + 4c_1 - c_2)\ /h/2 \qquad (A.$$
$$\gamma = (c_0 - 2c_1 + c_2)\ /h^2/2$$

Substitution into (A. 3) yields

$$A_{rest} = h[-c_0 + 8c_1 + 5c_2]/12 \qquad (A$$

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where it amounts + 0.23 l/min. This indicates that the values predicted from the analog computed curves agree well with those from the numerical calculation in the interval of cardiac outputs found during rest and mild exercise The correspondance between the two methods of integration is better than that reported by other authors (9)

In the clinical situation the analog computer here described should be considered a reliable instrument when dealing with normal dye dilution curves. It will give the cardiac output value within a minute of dye injection

The digital computation takes longer time to complete, but has the advantages of giving better control when abnormal curve forms occur It enhances the possibilities for providing diagnostic error notes and simultaneous calculation of other parameters like mean transit times, central vascular volumes, and curve variances (17) In our laboratory this method now is used routinely and has been incorporated into a more elaborate program system for the analysis of hemodynamic data from hypertensive patients. Compared with the manual method it is obviously time saving

Appendix

By Simpson's rule (12) the area under a curve passing through three points with equispaced abscissas is approximated by the area of a parabola passing through the same points. It is generalized to include an uneven number of points n, by summing the contribution from a series of such triplet points. With abscissa distance h between the points the formula is

$$A = (h/3)\ (c_1 + 4c_2 + 2c_3 + 4c_4 + \dots + 4c_{n-1} + c_n) \qquad (A\ 1)$$

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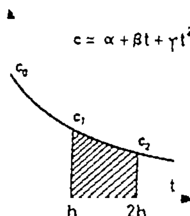


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Acta Med Scand Suppl. 603:23—28, 1977

Fifteen years of cardiac pacing

By Ole-Jørgen Ohm

reatment with permanent cardiac pacing arted in Medical Department A in 1962. uring the 15 years to 1976 a total number 336 patients have received this treatment shown in Figure 1 Compared with the st year of cardiac pacing when three new patients got permanent pacemakers, the number of patients with new pacemakers implanted has increased to 74 in 1976.

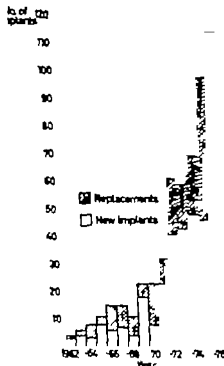


Fig 1

The author is now research fellow at The Chr Michelsen Institute Department of Science and Technology Nygaardsgt. 114 5000 Bergen, Norway

During the first years the pacemakerimplantations were mainly restricted to patients with high grade atrio-ventricular block (AVB) This picture has completely changed during the last few years (Table I) After 1971 there has been a gradual increase in the number of patients with atrial rhythm disturbances, especially the tachy-bradyarrhythmias and sino-atrial blocks. The last three years atrial arrhythmias and AVB comprise each half of the patients. The total number of new pacemaker implantations in patients with high grade AVB has also been increasing There may be several reasons for this increase. The wide use of the coronary care unit in our department has led to a more careful observation of patients with heart rhythm disturbances. Intermittent AVB and fascicular blocks (Table I) as a cause of dizziness and syncope have thereby been diagnosed in an increasing number In a Swedish material, obtained before the coronary care units came into use, Johansson (2) found an incidence of 6.3 patients per 100 000 inhabitants per year with complete heart block. Combining this with the data presented in Table I should indicate that an even higher number of patients with AVB still remains undiagnosed in our region, which covers a population of 500.000. Although we still do not implant pacemakers in patients with AVB or fascicular blocks who

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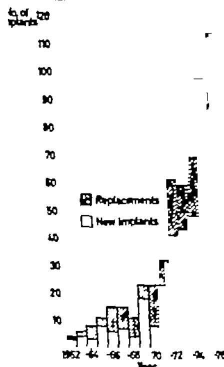


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Table 1 Dominant rhythm disturbance in 336 patients leading to pacemaker treatment

ARRHYTHMIAS	1962	63	64	-65	-66	67	-68	-69	70	71	72	73	74	-75
Sinus bradycardia										1	3	2	1	1
SA block and hyper sensitive carotid sinus							1	2		2	5	8	9	8
Coronary sinus and A V junctional bradyarrhythmias										1	3		3	2
Tachy bradyarrhythmias					1				1		3	8	7	6
Slow atrial fibrillation with and without complete A V block		1		2		1				1	1	2	6	3
A-V block I III	3	3	3	6	5	6	3	16	6	17	25	18	17	23
Intermittent A V block bi and trifascicular blocks									1	1	1	2	5	3
Ventricular fibrillation												1		
Asystole												2		
TOTAL	3	4	3	8	6	7	4	18	8	23	41	43	48	46

are symptom free, a better diagnostic procedure may result in a further increase in candidates for permanent pacemaker treatment. The development of the demand pacemaker which was introduced in our department in 1969 also caused an increase in the number of pacemakers being used. Better knowledge of the pacemaker treatment both among doctors and patients, and the understanding of a better quality of life for most of these patients, have led to a greater number of pacemaker implantations Of great importance is also the possibility of combining pacemaker treatment with digitalis and antiarrhythmic drugs which has proved most successful in patients with tachy bradyarrhythmias

Fixed rate versus demand systems

In general all patients with constant high grade AVB and slow idioventricular rhythm should be treated with fixed rate pacemaker This principle has been followed even for patients with coronary heart disease Although interference with spontaneous rhyt also will occur in patients with constant h grade AVB it has been strongly deba whether this mechanism may lead to ven cular tachyarrhythmias in this patient gro or it has at least been maintained that it occur as an exception only (11)

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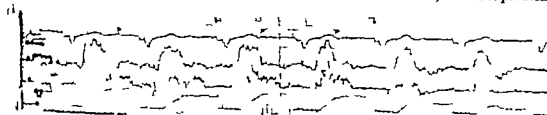
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Energy sources

The mercury zinc cells have been the most widely used energy source up to this date. Reducing internal energy consumption to a minimum and improving the cells has resulted in extension of battery life for fixed rate as well as demand units to 3–4 years and even more. Pulse generators with mercury zinc cells should therefore still be used in the oldest patients or in patients with reduced life expectancy due to complicating cardiac or other serious diseases.

In our patient group the age range is 4–91 years with a mean of 69.6 years. 40 patients (11.9%) are less than 60 years, while only 16 (4.8%) are less than 50 years of age. In the latter group are two patients with serious congenital heart disease.

The development of the new lithium powered pulse generators with an estimated battery life of more than 10 years has been promising. Further improvements of the lithium cells therefore may solve the problem



…g. 2 From patient with high grade atrio-ventricular …ock, aortic stenosis and insufficiency treated with …RS-inhibited pacemaker. The curves represent …om top to bottom): Standard lead I in ECG, mean …rtic blood-flow velocity, maximum aortic blood-flow …locity, integrated mean flow velocity. The con…ibution of atrial systole to the gross aortic flow output is clearly demonstrated by the distances between the various P-waves and the pacemaker triggered (↓) QRS-complexes (top curve) and the height of the peak forward integrated flow (bottom curve). The curves are recorded during maximal expiration. Paper speed 100 mm per second.

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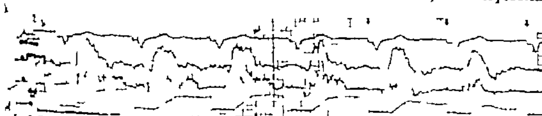


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of a lifetime pacemaker for most of the patients or at least with only one or two replacements. The need for a nuclear powered pacemaker seems not to be urgent at the moment.

Bipolar versus unipolar electrodes

The bipolar systems have some obvious advantages over the unipolar systems. In a series (8) covering the period 1967—1974 we used bipolar electrodes in 38 out of 213 cases (18%). For the bipolar electrodes the complication rate was low (5%) as compared with 31% for the unipolar systems. Stimulation of diaphragm was the most frequent complication and did not occur with the bipolar electrodes.

In addition bipolar demand systems are very little affected by external interference, there will be no stimulation of pectoral musculature and demand units will not be inhibited by myopotentials from skeletal muscles. In patients with dilated right ventricle bipolar electrodes are more easily placed in a stable position. Since there are two electrodes in contact with the endocardium the one with lowest threshold is used as the negative terminal irrespective of whether this is the distal or proximal pole. In the case of a fracture of one of the leads one has the possibility of making the system unipolar. A major disadvantage, however may be problems in obtaining an adequate demand function. This will especially apply in cardiac diseases with electrograms of low amplitudes.

Endocardial versus myocardial electrodes

Except for the first twelve pacemaker implantations, the transvenous route has always been used for permanent cardiac pacing as first choice. Only in four patients with exit block due to high threshold values or recurrent electrode displacements and demand failures it was impossible to establi quate pacemaker function. These pa were equipped with a myocardial syste a 4-year old boy with sinus arrest after an operation for correction of transpo of the great arteries a primary myoca implant was performed. The minor su procedure in endocardial implants is th favour of this method. The number of electrode displacements will be reduced specially trained surgeons or cardiol are performing the implantations (8).

There is no general agreement about to design the ideal electrode. In an effo reduce the number of displacements exit blocks, several electrode configura have lately come into clinical use. In add to the most commonly used endoca electrodes with flange tip different ver of hook electrodes have been designed (

Pulse generator and electrode

The use of miniaturized hybrid and i grated circuits have resulted in sm pulse generator dimensions, and the ch of new electronic components has red internal energy consumption to a minim

Reduced electrode area with conseque lower myocardial threshold values ma another important factor to prolong bat life. Energy dissipation has also been red by decreasing the pulse width from 1. down to about 0.5 ms in accordance the strength-duration curve for the he muscle. Different programmable pacem systems with the possibility of adju pulse width current drain and pacing r have been in clinical use for some years. own experience is limited to 20 patients these units powered by mercury zinc c So far none of the pulse generators has pa 39 months before being replaced.

Improvement and optimization of sensing function of demand pacemakers among the great challen es within p

ter technology. Proposals for standardiz n of test signals to examine the demand ction have been given, but are not gener accepted. Manufacturers have adopted ir own test pulses which are quite different n the intracardially measured signal. To ure an optimal match for QRS-sensing in S-inhibited and QRS-triggered pace kers and P waves in atrial triggered units, horough study of the signal properties of racardially recorded potentials is needed. ly with this approach the best correlation ween the test signal used and intra dially recorded signals can be found and ther improvement of the pulse generator put filters be achieved.

ectrochemical aspects of cardiac cing

ter an emitted pacemaker pulse polariza n phenomena are taking place in the tran ion zone between electrode and heart sue. Demand pacemakers sensing this er-potential may therefore be undesirably nibited. To avoid this a refractory period about 300 ms is incorporated in the system. te tissue impedance between the two pace aker electrodes has been measured to be proximately 500 ohms with endocardial ectrodes and accounts for much of the ectrode load impedance during heart stimu tion. Little is known, however about the ectrode signal source impedance which is major importance to an adequate demand nction. One may consider this impedance consisting of a series coupling between the sue resistance and a parallel coupling of e Faraday resistor and Helmholtz capacity 3) Investigations in our laboratory have own that the magnitude of the Faraday sistor is > 20 K ohms and the Helmholtz pacity lies in the region of 1—4 micro rads for permanently implanted electrodes 7) These values depend on the size of the lectrode used The smaller the electrode urface the higher the equivalent impedance.

For patients with a low amplitude electrogram, high output impedance in the heart matched with a pulse generator with low input impedance, a signal damping will be the result with a high risk of sensing failure. Since the source impedance increases at lower frequencies this effect will be even worse in patients with bundle branch block.

Future

The annual number of pacemaker implantations is expected to further increase In 1974 the number of new implants in Norway was 109 per one million inhabitants. Great geographical differences are found, implantation rates varying from 38 to 250 per million individuals per year in different areas. One may expect that the ratio between the number of replacements and the number of new implantations will be reduced with increasing pulse generator longevity (Fig 1)

For patients in high grade AVB and slow idioventricular rhythm one should still use fixed rate pacemakers as the first choice due to their insensitivity to external interference. For the remaining patients a ventricular demand pacemaker will be most appropriate. However In some patients it is recommendable to try atrial triggered, atrial, or perhaps atrio-sequential pacing although these systems are not widely used for the moment. They may prove of great value in temporary cardiac pacing in the critically ill patients with myocardial infarction or after open heart surgery

There is still much work to be done with regard to configuration both for atrial and ventricular electrodes. Although energy consumption has been reduced and myocardial threshold values decrease with the smaller electrodes, these electrodes should not be selected without reservation because of the risk of demand failure. Especially patients with low amplitude electrograms are inclined to develop demand failures due to entrance block.

of a lifetime pacemaker for most of the patients or at least with only one or two replacements. The need for a nuclear powered pacemaker seems not to be urgent at the moment.

Bipolar versus unipolar electrodes

The bipolar systems have some obvious ad vantages over the unipolar systems. In a series (8) covering the period 1967 —1974 we used bipolar electrodes in 38 out of 213 cases (18%) For the bipolar electrodes the com plication rate was low (5%) as compared with 31% for the unipolar systems Stimu lation of diaphragm was the most frequent complication and did not occur with the bipolar electrodes

In addition bipolar demand systems are very little affected by external interference there will be no stimulation of pectoral musculature and demand units will not be inhibited by myopotentials from skeletal muscles. In patients with dilated right ventricle bipolar electrodes are more easily placed in a stable position. Since there are two electrodes in contact with the endocardium the one with lowest threshold is used as the negative terminal irrespective of whether this is the distal or proximal pole. In the case of a fracture of one of the leads one has the possibility of making the system uni polar A major disadvantage, however may be problems in obtaining an adequate de mand function. This will especially apply in cardiac diseases with electrograms of low amplitudes.

Endocardial versus myocardial electrodes

Except for the first twelve pacemaker im plantations, the transvenous route has always been used for permanent cardiac pacing as first choice. Only in four patients with exit block due to high threshold values or recur rent electrode displacements and demand failures it was impossible to establi[sh ade]quate pacemaker function. These pa[tients] were equipped with a myocardial syste[m] a 4-year old boy with sinus arrest after h[is] an operation for correction of transpo[sition] of the great arteries, a primary myoc[ardial] implant was performed The minor m[...] procedure in endocardial implants is a[...] favour of this method. The number of [...] electrode displacements will be reduced [...] specially trained surgeons or cardiol[ogists] are performing the implantations (8)

There is no general agreement about [how] to design the ideal electrode. In an eff[ort to] reduce the number of displacements [and] exit blocks, several electrode configur[ations] have lately come into clinical use. In add[ition] to the most commonly used endocar[dial] electrodes with flange tip, different ver[sions] of hook electrodes have been designed (1

Pulse generator and electrode

The use of miniaturized hybrid and i[nte]grated circuits have resulted in sm[aller] pulse generator dimensions and the ch[oice] of new electronic components has red[uced] internal energy consumption to a minim[um]

Reduced electrode area with consequ[ent] lower myocardial threshold values mu[st be] another important factor to prolong bat[tery] life. Energy dissipation has also been red[uced] by decreasing the pulse width from 1.5 down to about 0 5 ms in accordance [with] the strength-duration curve for the he[art] muscle Different programmable pacema[ker] systems with the possibility of adjus[ting] pulse width current drain and pacing r[ate] have been in clinical use for some years. (own experience is limited to 20 patients [with] these units powered by mercury zinc c[ells] So far none of the pulse generators has pa[ssed] 39 months before being replaced

Improvement and optimization of sensing function of demand pacemakers among the great challenges un p[...]

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Intestinal adaptation

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We are still far from having the ideal pulse generator Size has diminished with weights < 80 grams for the smallest units, making them more suitable for children. Hermetical sealing to protect the electronic circuits from being destroyed by body fluid is certainly an improvement. Programmable pacemakers have been on the market these last 4 or 5 years with the possibility of non invasive adjustment of pacing rate, output and pulse width In future it may also be possible to alter sensitivity for incoming wave forms by use of an external programmer

A major task is to develop pacemaker systems for the treatment of patients with recurrent supraventricular and ventricular tachycardias caused by reentry mechanisms. A promising system has recently been described (10) Before implanting a permanent system it is always necessary to study with a temporary pacing unit whether pacing should be done from atrium or ventricle, and to investigate drug action on atrioventricular conduction.

An implantable system for automatic defibrillation of recurrent ventricular fibrillation has been developed (6) This system is now being tested in chronic animal implantations Demand pacemaking can be included in the design. This seems to be a promising device for patients with coronary heart disease fascicular blocks and recurrent episodes of ventricular fibrillation

An increasing number of patients with different types of cardiac diseases and rhythm disturbances are treated with permanent pacemakers. The number of types of pacemakers is increasing rapidly To cope with the many technical and medical problems a close cooperation between technicians and doctors is necessary There is need for a standardization of electrodes and electronic circuits together with a better knowledge of the technical specifications of the different pulse generators. All this is essential for a full understanding of the pacemaker function and only in this way it will be possible select the most suitable pacemaker for t[illegible] difficult cases.

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Intestinal adaptation

D. W Skagen and H. Schjønsby

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Experimental and clinical models

Resections and bypass-operations Resection or bypass of parts of the small intestine induces trophic changes in the remaining part (4 8, 24) These trophic changes include increased length and diameter of the intestine, increased villus height and crypt size and increased cell prodution. The size (13) and ultrastructure (29) of the cells lining the villi is not changed. Thus a pure hyperplasia occurs. The change is most prominent distal to the anastomosis. It is also dependent on the site of resection, being more prominent in the ileum after excluding a proximal part of the intestine than in the jejunum after resection or bypass of the ileum. Normally the proximal part of the intestine has the greatest absorptive surface and capacity Experiments which involved interchanging intestinal segments, revealed that the villus height was adapted to the actual distance from pylorus (4) In the excluded segment most observers have found the villus and crypt size as well as the cellular production reduced.

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Destruction of the mucosa In coeliac disease, there is a marked reduction in the life span of the villus epithelium This is to some extent compensated by an increased cell production (35) In some patients in whom the destruction is restricted to the jejenum increased ileal absorption of sodium and chloride (28) as well as vitamin B_{12} (22) has been observed. An increased cell production is also evoked in the intestinal mucosal surface when it is explanted to the abdominal wall (20)

After ischemia of the small intestine for 1—2 hours, the villus epithelium is selectively destroyed This is rapidly followed by a markedly increased cell production (25) Irradiation, on the other hand preferentially destroys the rapidly proliferating cells in the crypts At a proper dosage, only a few proliferative cells will be intact while the villus epithelium apparently is unaffec ted Also here, an intense regeneration ensues, but not before the villus epithelium is ex truded after having reached its normal age (26)

Finally the absence of bacteria in the lumen has been shown to decrease villus size and cell production (1) while increased villus and crypt size is found in the blind loop syndrome (27)

Alterations in food intake In starvation a substantial decrease in the content of water protein and DNA in the total small intestine occurs already after 24 hours (31) The reduction of water and protein is greater in the intestine than in other organs and also exceeds the reduction of DNA Simul taneously cell production is decreased (2)

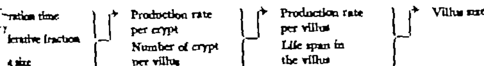
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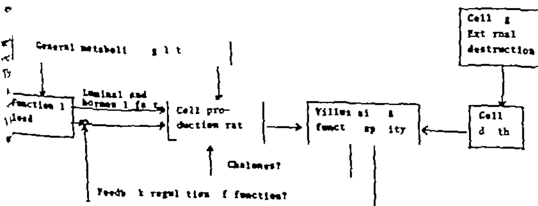
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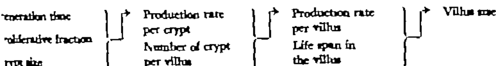
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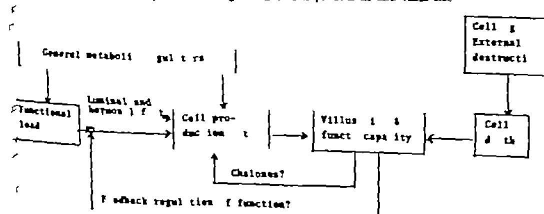
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The generation time which is the ti interval from a cell is formed by a divisi until it divides itself is generally shorten when the production rate is increased a prolonged when it is decreased (2) This most marked when the cell production rate abruptly changed When a new steady st has been established after a prolonged infl ence the generation time becomes near normal (23) while the crypt size and numb of crypts per villus are changed The gene ation time is further divided into four co secutive phases G_1 which is a postmitot 'resting' phase S when the DNA repl cation takes place G_2 when the cell prepar for mitosis, and M which is the mitosis. Gene aly G_1 is the most flexible and the princip effect of an acute stimulus is to induce cel in G_1 to enter S These cells can be followe

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as described earlier It has also been found that adaption to resection fails to occur or is weakened if the animal has only been nourished intraveneously (10 11) The intraluminal factors may be components in the food or substances in the secretions of the upper gastrointestinal tract. Altmann (3) demonstrated trophic factors in the pancreatic juice Later others (14) have found a direct trophic effect of food components, which is partly inhibited by pancreatic juice and bile. This discrepancy may be due to difficulties in separating the direct effect of pancreatic secretions from its digestive effect on the food.

Several findings also point to the influence of gastrointestinal hormones Gastrin has been shown to have a trophic effect, both in vivo and in vitro (19) Among the other gastrointestinal hormones CCK PZ seems to have little influence on the small intestinal cell proliferation (16) Secretin does however seem to antagonize the trophic action of gastrin (15) Furthermore it has been found that the responses to starvation (6) and lactation (9) also occur although to a lesser degree, in segments out of contact with the chyme Finally Gleeson (12) described a patient with a tumor secreting enteroglucagon who had villus hypertrophy The patient also had a stagnant loop syndrome however

In conclusion, present knowledge indicates two major regulatory mechanisms which determine the adaptation to changing conditions in the small intestine. One is the reparative response to villus cell destruction, the other the stimulatory effect of food components in the chyme.

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The mechanism of lincomycin induced diarrhoea

Stein O. Hoff, Henning Schjønsby and Knut Jan Andersen

STRACT Metabolic studies were performed be- and seven days after treating rats orally with comycin. Following the treatment the mean faecal ight increased from 50.2 g/72 hr ± 5.8 (S.D.) to .5 ± 8.2. The faecal fat excretion was unchanged, d the weight increase was mainly due to increased ater content. T find whether the watery diarrhoea s due to bile acid malabsorption, the absorption te of [^{14}C]-taurocholic acid was measured in un- ated rats and rats treated with lincomycin using an vivo perfusion technique. There was no significant ference in bile acid absorption rate measured at ree different concentrations of bile acid in the rfusate. Alternative mechanisms of lincomycin- induced diarrhoea are discussed.

During the past 9 years and particularly since 1975 numerous reports have shown that treatment with lincomycin and its derivative clindamycin may cause diarrhoea and pseudomembranous colitis (1 6 8, 9 1 13, 16, 18, 20 21). Different publications report an incidence of diarrhoea varying from to 20 / (1 9 20 21). The pathogenesis of the diarrhoea is poorly understood. A number of theories exist, but no conclusive evidence of either bacterial overgrowth (13 16, 18) hypersensitivity reaction (6) or direct toxic action of the antibiotic or its metabolites (8, 14) has yet been produced. In pseudomembranous colitis different workers claim to be able to demonstrate fibrin thrombi (2) infarction of the bowel (17) and particles suggestive of a viral disease (12 19). It is, however not certain that the same mechanism is responsible for the severe colitis as for the simple diarrhoea although the former may be the result of a seemingly straight forward progression from the latter.

Burbige and Milligan (3) reported that two patients with diarrhoea and pseudomembranous colitis were successfully treated with cholestyramine and suggested that the diarrhoea was due to bile acid malabsorption. Furthermore we found the absoption coefficient of [1 ^{14}C]-glycocholic acid reduced after lincomycin treatment in four out of seven patients with continent ileostomy (Schjønsby et al. Unpublished).

This study was undertaken to test whether lincomycin-induced diarrhoea is due to bile acid malabsorption. This was investigated by measuring the absorption of [^{14}C] labelled taurocholic acid after perfusion of the bile acid in an ileal segment in both untreated rats and rats treated with lincomycin.

MATERIALS AND METHODS

Experimental animals. Male albino rats (Wistar strain) weighing 220—260 g were used for the perfusion studies. During the metabolic studies the weights were 283—319 g

Metabolic studies. Five rats were given food from the same batch of a commercial ground pellet diet and tap water. Urine and faeces were collected separately in metabolic cages. Water and food intake was estimated by daily weighing food cups and measuring volume of the drinking flasks. The first period of metabolic measurements lasted 72 hours during which the animals were untreated. Subsequently lincomycin chloride (1 mg/ml) was added to the drinking water for a period of 10 days. From the beginning of the 8th day of this treatment measurements of intake and excretion were repeated over another 72-hour period. The water content of the faeces was estimated by

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The mechanism of lincomycin induced diarrhoea

Stein G Holl, Henning Schjønsby and Knut Jan Andersen

ABSTRACT Metabolic studies were performed before and seven days after treating rats orally with lincomycin. Following the treatment the mean faecal weight increased from 30.2 g/72 hr ± 3.8 (S.D.) to 53.5 ± 8.2. The faecal fat excretion was unchanged, and the weight increase was mainly due to increased water content. To find whether the watery diarrhoea was due to bile acid malabsorption, the absorption rate of [^{14}C]-taurocholic acid was measured in untreated rats and rats treated with lincomycin using an in vivo perfusion technique. There was no significant difference in bile acid absorption rate measured at three different concentrations of bile acid in the perfusate. Alternative mechanisms of lincomycin-associated diarrhoea are discussed.

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drying at 100°C in a heating cupboard overnight. The fat content of the food and the faeces was measured by van de Kamer's method (22) and the fat absorption coefficient was calculated.

Bile acid perfusion. The bile acid absorption studies were carried out in vivo by perfusion of the small intestine essentially as described by Schiff et al (15) The rats were anesthetized with pentobarbitone. A 10 cm segment of the ileum, terminating approximately 10 cm from the ileocoecal junction was catherized in either end and flushed clear with 0 15 M NaCl and the abdomen was closed with sutures. A solution of [14C] labelled taurocholic acid (37°C) was then perfused at a rate of 0.5 ml/min. Bile was collected through a catheter directly into counting vials at 3 min intervals. The rate of appearance of the labelled bile acid became constant after 10 min and bile was collected in five 3-min samples starting after 15 min. Subsequently a second solution with a higher concentration of taurocholic acid was perfused. Body temperature was kept at approximately 37°C during the perfusion using a heat lamp.

At the end of the perfusion biopsies were obtained from the ileum and the colon for histological studies. The isolated segment was removed and the length was measured using a standard stretch of 5 g The [14C-] label in bile was determined by scintillation counting After internal standard correction the results were expressed as picomol taurocholic acid absorbed per min per cm length of intestine.

In initial experiments when a non-absorbable marker polyethylene glycol, had been added to the perfusate we showed that the amount excreted through the bile was the same as that lost in the per fusion fluid. Furthermore hepatic transport capacity of [14C] labbelled bile acids is known to be about five times higher than maximal intestinal absorption rates (7) Hence the appearance of labelled bile acids in the bile is an accurate measure of the rate of intestinal absorption.

Reagents Synthetic Taurocholic Acid, Sodium was obtained from Sigma Chemical Company Louis, USA, and Tauro [carbonyl-14C] cholic sodium salt, specific activity > 50 mCi/mmol the Radiochemical Centre, Amersham, UK. purity of these preparations was tested by thin chromatography (10) The perfusates were by dissolving varying amounts of the pure bile together with constant amounts of the radi tracer in Krebs-Ringer phosphate buffer (pH = 7

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Table I *Results of metabolic studies before and after lincomycin therapy (5 animals) The results are given as means ± standard deviation.*

	Before	After	p-value
Total faecal weight (g/72 h)	30.2 ± 3 8	65.5 ± 8.2	<0.001
Faecal dry weight (g/72 hr)	10 7 ± 1 8	18.3 ± 2 7	<0.01
Faecal water (g/72 hr)	19.5 ± 3.9	47.2 ± 5.8	<0.001
Fat excretion (g/72 hr)	0.41 ± 0 08	0 43 ± 0 03	n. s.
Fat absorption coefficient (%)	78 0 ± 2 6	83 6 ± 2 1	<0.01
Food intake (g/72 hr)	48.6 ± 6.5	68 0 ± 5 1	<0 01
Water intake (g/72 hr)	87.8 ± 10.6	143 0 ± 10 1	<0 001
Urinary volume (ml/72 hr)	28.8 ± 12 0	28 2 ± 6 9	
Urinary weight (g/72 hr)	32.6 ± 10 0	31 0 ± 7 0	

Table II. The absorption rate of [^{14}C]-taurocholic acid in untreated rats and rats treated orally with lincomycin. The results are given as means ± standard deviation. The number of experiments is shown in brackets

Bile acid concentration (mmol/l)	Absorption (pmol.min^{-1} cm^{-2}) Untreated rats	Lincomycin-treated rats
0.125	1171 ± 493 (5)	1350 ± 254 (6)
0.500	3940 ± 913 (5)	4269 ± 1068 (8)
1.500	10539 ± 2853 (6)	11062 ± 1953 (7)

significant difference between the absorption rates in untreated and lincomycin treated animals.

Histological studies by light microscopy of the ileum and the colon did not reveal any structural damage.

Diacussion

The metabolic studies show that the rats given lincomycin developed increased faecal weight. This increase was found both in dry faecal weight (71 %) and in water content (142%). The increased excretion was compensated by increased food (40 %) and water intake (63%). Although the fat absorption coefficient showed a small increase, the faecal fat excretion was unaltered. The changes thus indicate that rats develop watery diarrhoea on ingesting lincomycin.

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We did not find any microscopic evidence of structural damage and suggest in keeping with the hypothesis of Pittman et al (14) that functional derangement of absorbing cells explain the diarrhoea. Such derangement could be caused by the toxic effect of lincomycin or its metabolites. This antibiotic exerts its antibacterial action through inhibiting protein synthesis by blocking the enzyme ribosomal tranferase (4, 5). It is possible that this antimetabolic effect is also exhibited in the intestinal epithelium.

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Bile acid perfusion. The bile acid absorption studies were carried out in vivo by perfusion of the small intestine essentially as described by Schiff et al (15) The rats were anesthetized with pentobarbitone. A 10 cm segment of the ileum, terminating approximately 10 cm from the ileocoecal junction, was catherized in either end and flushed clear with 0 15 M NaCl and the abdomen was closed with sutures. A solution of [14C] labelled taurocholic acid (37°C) was then perfused at a rate of 0.5 ml/min. Bile was collected through a catheter directly into counting vials at 3 min intervals. The rate of appearance of the labelled bile acid became constant after 10 min and bile was collected in five 3-min samples starting after 15 min. Subsequently a second solution with a higher concentration of taurocholic acid was perfused. Body temperature was kept at approximately 37°C during the perfusion using a heat lamp.

At the end of the perfusion biopsies were obtained from the ileum and the colon for histological studies. The isolated segment was removed and the length was measured using a standard stretch of 5 g The [14C-] label in bile was determined by scintillation counting After internal standard correction the results were expressed as picomol taurocholic acid absorbed per min per cm length of intestine.

In initial experiments when a non-absorbable marker polyethylene glycol, had been added to the perfusate we showed that the amount excreted through th bile was the same as that lost in the per fusion fluid. Furthermore hepatic transport capacity of [14C]-labbelled bile acids is known to be about five times higher than maximal intestinal absorption rates (7) Hence the appearance of labelled bile acids in the bile is an accurate measure of the rate of intestinal absorption.

Reagents. Synthetic Taurocholic Acid, Sodium Sa was obtained from Sigma Chemical Company, S Louis, USA, and Tauro [carbonyl-14C] cholic ac sodium salt, specific acitivity > 50 mCi/mmol fro the Radiochemical Centre, Amersham, UK. Th purity of these preparations was tested by thin-lay chromatography (10) The perfusates were prepar by dissolving varying amounts of the pure bile ac together with constant amounts of the radioacti tracer in Krebs-Ringer-phosphate buffer (pH = 7.25

Statistical methods Experimental groups were com pared by the Student t test. Results were taken to b significant if the value of t correspond to the 5% significance level or better

Results

Metabolic studies The results of metabolic studies before and after oral treatment with lincomycin are shown in table I Following the treatment increased faecal weight was observed This was due mainly to an increase in the water content but also to an increase in the dry weight. The intake of water and food also increased These changes were seen in every animal tested. Neither urinary excretion nor faecal fat content showed any significant change, whereas there was a slight rise in the fat absorption coefficient.

Bile acid absorption. Absorption rates for [14C] taurocholic acid were estimated at varying concentrations of the bile acid in the perfusate in both groups of animals. The results of these perfusion experiments using three different concentrations of the bile acid are given in table II There was no

Table I *Results of metabolic studies before and after lincomycin therapy (5 animals) The results are given as means ± standard deviation.*

	Before	After	p-value
Total faecal weight (g/72 h)	30.2±3 8	65.5±8 2	<0.001
Faecal dry weight (g/72 hr)	10 7±1 8	18.3±2 7	<0.01
Faecal water (g/72 hr)	19.5±3 9	47.2±5 8	<0.001
Fat excretion (g/72 hr)	0 41±0 08	0 43±0.03	n. s.
Fat absorption coefficient (%)	78 0± 6	83 6±2 1	<0.01
Food intake (g/72 hr)	48.6±6 5	68.0±5 1	<0 01
Water intake (g/72 hr)	87.8±10 6	143 0±10 1	<0.001
Urinary volume (ml/72 hr)	28.8±12 0	28.2±6 9	n. s.
Urinary weight (g/72 hr)	32.6±10.0	31 0±7 0	n.s.

Table II. The absorption rate of [^{14}C] -taurocholic acid in untreated rats and rats treated orally with lincomycin. The results are given as means ± standard deviation. The number of experiments is shown in brackets

Bile acid concentration (mmol/l)	Absorption (pmol.min^{-1} cm^{-1})	
	Untreated rats	Lincomycin-treated rats
.125	1171± 495 (5)	1350± 254 (6)
0.500	3949± 913 (5)	4269±1088 (8)
1.500	10539±2853 (6)	11062±1933 (7)

significant difference between the absorption rates in untreated and lincomycin treated animals.

Histological studies by light microscopy of the ileum and the colon did not reveal any structural damage.

Discussion

The metabolic studies show that the rats given lincomycin developed increased faecal weight. This increase was found both in dry faecal weight (71%) and in water content (142%). The increased excretion was compensated by increased food (40 /) and water intake (63%). Although the fat absorption coefficient showed a small increase, the faecal fat excretion was unaltered. The changes thus indicate that rats develop watery diarrhoea on ingesting lincomycin.

The in vivo measurements of taurocholic acid absorption from the distal ileum did not, however show any difference in the two groups. This appears to exclude the hypothesis that bile acid malabsorption is the cause of the uncomplicated lincomycin-associated diarrhoea. Such a mechanism is also unlikely to operate to any important degree in the much more fulminant picture of pseudomembranous colitis. A back-wash ileitis, however like that seen in severe ulcerative colitis, might also be found in pseudomembranous colitis and explain bile acid malabsorption as a secondary phenomenon. This might account for the improved diarrhoea following cholestyramine (3). Alternatively the effect of cholestyramine could be due to binding of the antibiotic or its metabolites.

We did not find any microscopic evidence of structural damage and suggest in keeping with the hypothesis of Pittman et al (14) that functional derangement of absorbing cells explain the diarrhoea. Such derangement could be caused by the toxic effect of lincomycin or its metabolites. Thus antibiotic exerts its antibacterial action through inhibiting protein synthesis by blocking the enzyme ribosomal tranferase (4 5). It is possible that this antimetabolic effect is also exhibited in the intestinal epithelium.

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Osteomalacia in the stagnant loop syndrome

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ABSTRACT Osteomalacia in 80-year old woman with malabsorption due to the stagnant loop syndrome is reported. The osteomalacia was associated with bacterial overgrowth in the small intestine and increased bile salt deconjugation. Although the mechanism of osteomalacia in the stagnant loop syndrome remains uncertain, it is suggested that abnormal flora reduce the absorption of vitamin D by deconjugation of bile salts in the lumen of the small intestine

Osteomalacia is a rare complication of the stagnant loop syndrome (4) The purpose of the paper is to describe the clinical history of a patient with the stagnant loop syndrome, who gradually became disabled due to severe osteomalacia.

Clinical history

The patient (I T) was healthy until 1966, when she at the age of 70 years developed macrocytic anaemia. She was treated successfully with vitamin B_{12}-injections. 1 October 1971 she was admitted to the hospital for the first time due to diarrhoea and abdominal pain. Her weight was 41 kg and height 161 cm. The abdomen was meteoristic and there were oedemas in both legs. The hemoglobin was 12.6 g/100 ml. The serum cholesterol was 140 mg/100 ml, protein 5.3 g/100 ml and albumin 2.4 g/100 ml.

Absorption studies Absorption tests showed Schilling test of 0%/24 h when radioactive vitamin B_{12} was given without intrinsic factor and 0.9%/24 hr when the radioactive vitamin was given with intrinsic factor The faecal fat excretion was 4.0 g/24 hr and the 4 hour serum concentration of vitamin A after an oral test dose (250.000 i.u.) was 44 iu/100 ml (N > 80) The 5 hour urinary excretion of d-xylose after 25 g oral dose was 4.3 g The oral glucose tolerance test showed slightly elevated curve with the following glucose values in the venous blood fasting 70 mg/100 ml; 1 hour 185 mg/100 ml; 2 hours, 116 mg/100 ml 2½ hours, 63 mg/100 ml. The urinary excretion of indican was 296 mg/24 hr (N > 100) Quantitative bacteriological studies were performed on an aspirate from the upper jejunum. The concentration of aerobic microorganisms was 10^{10} microorganisms per ml and of anaerobic microorganisms 10^{10} per ml. A radiological study of the small intestine after a barium meal showed multiple and large diverticula in the duodenum and the jejunum. There was delayed passage through the small intestine and the front of the barium meal first appeared in the colon after 8 hours. Histological study of biopsy from the proximal part of the jejunum showed normal villus architecture.

The association of malabsorption with diverticula of the small intestine and bacterial overgrowth was suggestive of stagnant loop syndrome The results of oral antibiotic treatment confirmed the diagnosis. The patient was given oral treatment with lincomycin hydrochloride (2 g daily in divided doses for 4 days) Following the treatment, the Schilling test increased to 6%, and the faecal fat excretion decreased to 0.5 g/24 hr The urinary excretion of indican diminished to 193 mg/24 hr and quantitative bacteriological studies showed no growth of anerobic bacteria in the jejunum, whereas the concentration of aerobic bacteria was $5 \cdot 10^{6}$ per ml.

1 January 1972 the patient was readmitted due to relapse of the diarrhoea. The maximum acid output after pentagastrin stimulation was 0 meqv/hr After Lundh meal the concentration of amylase in the duodenal aspirate was 46 u/ml (N > 100) A plain X-ray of the abdomen did not show any pancreatic calcifications. A pancreatic enzyme preparation (Combizym®) was without effect, whereas oral treatment with tetracyclin promptly stopped the diarrhoea.

Development of osteomalacia. 1 March 1972 the patient developed pain in the lower back. X-ray studies of the lumbar spine showed osteoporosis and compression fractures of the first, second and third lumbar vertebral bodies. Serum calcium was 8.7 mg/100 ml, phosphate 3.6 mg/100 ml, alkaline phosphatase 148 B& L u/100 ml (N < 90) and gammaglutamyl trans-

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ferase 11 u/l (N<50) A bone biopsy was obtained from the ileal crest. Histological studies showed osteomalacia with increased thickness of the osteoid seam (G Bang M D., The Gade Institute, University of Bergen) Following an intravenous dose of 40 000 I u. vitamin D_2 (7) the serum phosphate increased to 4.3 mg/100 ml.

During the subsequent years the patient was given regular vitamin B_{12} injections and oral treatment with 60.000 I.u. vitamin D_2 and 1 g calcium lactate daily In March 1974 there was abdominal distension and pain in the back. Serum calcium was 8.8 mg/100 ml, phosphate 2 7 mg/100 ml and alkaline phosphatase 127 B& L u/100 ml Urinary indican excretion was 178 mg/24 hr The "breath test indicated increased bile salt deconjugation After an oral dose of [1 ^{14}C] glycine-glycocholic acid the cumulative $^{14}CO_2$-expiration was 11 1%/8 hr (N<3 7%) Following oral treatment with tetracyclin for 4 days, indican excretion decreased to 37 mg/24 hr whereas serum phosphate increased to 3 9 mg/100 ml and calcium to 9.2 mg/100 ml.

In 1976 the clinical condition gradually deteriorated. She was readmitted in February 1976 with a relapse of the diarrhoea. Her height had decreased to 151 cm and the Schilling test was 0%/24 hr Following an oral course with lincomycin there was symptomatic improvement and the Schilling test increased to 11% In May she was given an oral course with metronidazol because of relapse of the diarrhoea. In June the patient became increasingly immobile due to oedemas and pain in the back and the legs. A X ray study showed a fracture in the left femoral collum without dislocation. She was treated with physiotherapy and given crutches. She is still unable to walk about without the crutches.

In October she was readmitted as a result of relapse of the bone pain and oedemas. There was marked kyphosis and the lower ribs were resting on the ileal crests due to collapse of the vertebral bodies. The abdomen was distended and the height of the patient was 148 cm. Serum albumin was 2 6 g/100 ml and total protein 6 1 g/100 ml Serum folate was 20 ng/ml and cholesterol 121 mg/100 ml. The urine was proteinfree, the serum creatinine 0 6 mg/100 ml. Urinary excretion of calcium was 45 mg/24 hr while the phosphate excretion was 300 mg/24 hr Serum parathormone was 170 pmoleeqv/l (N 120 – 260) phosphate 3.2 mg/100 ml, the calcium 8 9 mg/100 ml alkaline phosphatase 1066 u/l (N<270) Bromsulphalein retention after 45 minutes was 1.5 and all other liver function tests were normal. A biopsy obtained from the ileal crest showed osteomalacia with a greatly increased thickness of the osteoid seam Radiological studies showed markedly decreased bone density and symmetrical Milkmann fractures on the inner aspect of the upper humerus and the proximal phalanges of the second, third and fourth finger

Absorption studies showed a faecal fat excretion of 25 g/24 hr and a Schilling test value of 1 %/24 hr $^{14}CO_2$-expiration after an oral dose of ^{14}C glycocholin was 19 4%/8 hr The patient was treated with lincomycin and the Schilling test increased to 6.4%.

After discharge from the hospital the patient has been unable to move out of her home She is dependent on a social home help to do most of the house work. Since oral treatment with vitamin D clearly was insufficient, a nurse visits her home at weekly intervals to give her injections with vitamin D

Discussion

The radiological features of decreased bone density and Milkmann fractures were strongly suggestive of osteomalacia and this diagnosis was confirmed by the ileal crest biopsies. The biochemical findings also support the diagnosis since there was elevated alkaline phosphatase Decreased serum phosphate is also a feature of ostemalacia In the patient most serum phosphate measurements were normal, but on three occasions the phosphate values were 2 7 2 8 and 2 9 mg/100 ml. ml The serum calcium was within the normal range on repeated occasions According to Salvesen and Boe (1) serum calcium is frequently normal in patients with osteomalacia and sprue and these patients also develop secondary hyperparathyreoidism. The extent to which secondary hyperparathyreoidism was present in this patient is, however uncertain Some degree of osteoporosis is also likely as a result of old age and increasing inactivity

The mechanism of osteomalacia in the stagnant loop syndrome is uncertain Since the patient developed progressive osteomalacia in spite of oral supplements with vitamin D it is likely that malabsorption of vitamin D played an important role Malabsorption of vitamin D was also demonstrated in two patients with osteomalacia and the stagnant loop syndrome (3 6)

Since bacterial overgrowth in the small

bowel is capable of interfering with the absorption of several substances including fat (4) it is feasible that bacteria play a role in the malabsorption of vitamin D The role of bacteria is also supported by the increase in serum phosphate from 2.7 to 3.9 mg/100 ml after oral treatment with tetracyclin. Similar results were found in another patient (E J) who was admitted to our department in 1968 suffering from a stagnant loop syndrome (2) The serum phosphate was 1 7 mg/100 ml and calcium 8.3 mg/100 ml. After 15 days of tetracyclin therapy the phosphate was 4 0 mg/100 ml and calcium 8.3 mg/100 ml.

Malabsorption of vitamin D in the stagnant loop syndrome could be a result of damage to the enterocyte or impaired micellar formation due to deficiency of conjugated bile salts. Alternatively it could be caused by bacterial uptake of vitamin D in competition with the host. Decreased pancreatic function also is a possibility (5) Apart from a reduced concentration of amylase in the duodenum on a single occasion there was no evidence of pancreatic disease. Furthermore osteomalacia is only rarely seen in chronic pancreatic insufficiency In this patient there was no evidence of damage to the enterocyte, whereas the result of the breath test suggested abnormal bile salt deconjugation. Increased bile salt deconjugation in the stagnant loop syndrome frequently results in deficiency of conjugated bile salts and impaired micellar formation (4) Sèze and coworkers (3) found decreased levels of conjugated bile salts after a meal in the jejunum of a patient with osteomalacia and stagnant loop syndrome although they could not exclude cholestasis as a cause of this decrease. Obviously further observations are needed to find whether malabsorption of vitamin D in the stagnant loop syndrome is due to increased deconjugation of bile salts.

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ferase 11 u/l (N<50) A bone biopsy was obtained from the ileal crest. Histological studies showed osteomalacia with increased thickness of the osteoid seam (G Bang M D The Gade Institute, University of Bergen) Following an intravenous dose of 40 000 I.u. vitamin D_3 (7) the serum phosphate increased to 4 3 mg/100 ml.

During the subsequent years the patient was given regular vitamin B_{12} injections and oral treatment with 60 000 I.u. vitamin D_2 and 1 g calcium lactate daily In March 1974 there was abdominal distension and pain in the back. Serum calcium was 8.8 mg/100 ml, phosphate 2 7 mg/100 ml and alkaline phosphatase 127 B& L u/100 ml. Urinary indican excretion was 178 mg/24 hr The breath test indicated increased bile salt deconjugation. After an oral dose of [1-^{14}C] glycine-glycocholic acid the cumulative $^{14}CO_2$-expiration was 11 1%/8 hr (N<3 7%) Following oral treatment with tetracyclin for 4 days, indican excretion decreased to 37 mg/24 hr whereas serum phosphate increased to 3 9 mg/100 ml and calcium to 9.2 mg/100 ml.

In 1976 the clinical condition gradually deteriorated She was readmitted in February 1976 with a relapse of the diarrhoea. Her height had decreased to 151 cm and the Schilling test was 0%/24 hr Following an oral course with lincomycin there was symptomatic improvement and the Schilling test increased to 11% In May she was given an oral course with metronidazol because of relapse of the diarrhoea. In June the patient became increasingly immobile due to oedemas and pain in the back and the legs. A X ray study showed a fracture in the left femoral collum without dislocation. She was treated with physiotherapy and given crutches. She is still unable to walk about without the crutches.

In October she was readmitted as a result of relapse of the bone pain and oedemas. There was marked kyphosis and the lower ribs were resting on the ileal crests due to collapse of the vertebral bodies. The abdomen was distended, and the height of the patient was 148 cm. Serum albumin was 2 6 g/100 ml and total protein 6.1 g/100 ml. Serum folate was 20 ng/ml and cholesterol 121 mg/100 ml. The urine was proteinfree the serum creatinine 0 6 mg/100 ml. Urinary excretion of calcium was 45 mg/24 hr while the phosphate excretion was 300 mg/24 hr Serum parathormone was 170 pmoleeqv/l (N 120–760) phosphate 3.2 mg/100 ml, the calcium 8.9 mg/100 ml, alkaline phosphatase 1066 u/l (N<270) Bromsulphalein retention after 45 minutes was 1.5 and all other liver function tests were normal. A biopsy obtained from the ileal crest showed osteomalacia with a greatly increased thickness of the osteoid seam. Radiological studies showed markedly decreased bone density and symmetrical Milkmann fractures on the inner aspect of the upper humerus and the proximal phalanges of the second, third and fourth finger

Absorption studies showed a faecal fat excretion of 25 g/24 hr and a Schilling test value of 1%/24 hr $^{14}CO_2$-expiration after an oral dose of ^{14}C glycocholin was 19 4%/8 hr The patient was treated with lincomycin and the Schilling test increased to 6.4%.

After discharge from the hospital the patient has been unable to move out of her home. She is dependent on a social home help to do most of the house work. Since oral treatment with vitamin D clearly was insufficient, a nurse visits her home at weekly intervals to give her injections with vitamin D

Discussion

The radiological features of decreased bone density and Milkmann fractures were strongly suggestive of osteomalacia and this diagnosis was confirmed by the ileal crest biopsies. The biochemical findings also support the diagnosis since there was elevated alkaline phosphatase Decreased serum phosphate is also a feature of ostemalacia. In the patient most serum phosphate measurements were normal but on three occasions the phosphate values were 2 7 2 8 and 2 9 mg/100 ml. ml The serum calcium was within the normal range on repeated occasions. According to Salvesen and Boe (1) serum calcium is frequently normal in patients with osteomalacia and sprue and these patients also develop secondary hyperparathyreoidism. The extent to which secondary hyperparathyreoidism was present in this patient is, however uncertain. Some degree of osteoporosis is also likely as a result of old age and increasing inactivity

The mechanism of osteomalacia in the stagnant loop syndrome is uncertain Since the patient developed progressive osteomalacia in spite of oral supplements with vitamin D it is likely that malabsorption of vitamin D played an important role Malabsorption of vitamin D was also demonstrated in two patients with osteomalacia and the stagnant loop syndrome (3 6)

Since bacterial overgrowth in the sm

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Intensive treatment in severe acute attacks of ulcerative colitis

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ABSTRACT The results of intensive treatment including steroids and early surgery in severe colitis in the period 1971–1975 were compared to the results from the period 1966–1970 when systematic intensive therapy was not given. The period of preoperative medical treatment was reduced on average from 31 to 17 days. Approximately half the patients went into remission in each period. In the period 1971–1975 (23 patients) there was no mortality during the acute attacks or in the subsequent follow-up period. In the period 1966–1970, the immediate mortality in 30 patients was 10% (3 patients) One patient died six years later at the age of 81 during relapse of the colitis, and the overall mortality was 13.3%

In patients with severe attacks of ulcerative colitis there is a high fatality rate (2) Remissions can be encouraged by using corticosteroids (9) and possibly also by intensive intravenous therapy (10) Nevertheless the use of steroids in acute attacks is still being debated, as the overall mortality in patients on steroid treatment appears to be high (6 10) Early surgery in patients who fail to improve on conservative treatment has also been proposed to reduce the fatality rate (4 5)

Early in 1971 a closer form for cooperation was carried out between the departments of Medicine Surgery and Anaesthesiology in the treatment of severe ulcerative colitis. We adopted a policy of intensive intravenous therapy including steroids and early surgery in patients who failed to go into remission We present here the results from a 5-year trial period.

PATIENTS AND METHODS

During 10-year period from 1 January 1966 to 31 December 1975 148 patients with ulcerative colitis were admitted to the hospital. 53 patients had acute severe colitis. 30 patients with severe attacks were admitted in the 5-year period before 31 December 1970, and 23 patients in the 5-year period from 1971 The age distribution is recorded in table I and the extent of involvement of the large bowel is shown in table II. The diagnosis and the extent of the disease was established by sigmoidoscopy barium enema, and in the operated patients by inspection and pathological examination of the resected specimens. The length of the history of the disease before the severe attack was nearly the same in the period from 1971–1975 (mean 1 6 years, range 2 weeks – 9 years) as in the period from 1966–1970 (mean 2 years, range 1 week – 8 years)

Table I *Age of patients with severe ulcerative colitis*

Age (years)	1966–1970 (no)	1971–1975 (no)
0–19	11	6
20–39	10	9
40–59	5	6
60–	4	2
Mean age	33.0	32.1

Table II. *Extent of involvement of bowel*

Extent	1966–1970 (no)	971–1975 (no)
Distal (rectum and sigmoid)	0	1
Intermediate	10	5
Total	19	16
Unknown	1	1

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Table V. *Long term results in patients going into remission. Mean length of follow-up: Period 1966–1970 8.4 years (range 4.7–11.6); period 1971–1975, 3.9 years (1.6–5.7)*

Result	1966–1970 (no)	1971–1975 (no)
Remission	6	2
Relapses medically treated	5	7
Elective surgery	3	3
Unknown	1	0

first severe attack. She died one week after the operation due to peritonitis and septicemia.

The long-term fate of patients followed up after going into remission on medical therapy is summarized in table V. In the period 1966–1970 one patient died during a relapse of the colitis six years after the severe attack at the age of 81. The overall mortality in patients admitted with severe colitis in the period 1966–1970 was thus 13.3%. Of the patients admitted in the period 1971–1975 none have died during the subsequent follow-up period.

Discussion

The results show reduced mortality in the period 1971–1975 compared to the period 1966–1970. The change in overall mortality was mainly due to reduced operative mortality. In the first period the operative mortality in 15 patients was 20%, whereas none of 11 patients died after surgery in the second period. This fall in mortality was associated with a more intensive intravenous therapy and with a reduction in the time from onset of the attack until surgery. Each of these factors may have influenced the mortality results. There was however a limited number of patients and the difference in mortality could be due to chance.

About half of the patients went into remission in each of the two periods. This is a similar proportion of success of medical treatment as reported elsewhere (5, 6, 10). In the second period, corticosteroids were given to a larger proportion than in the first period, and total parenteral nutrition was given routinely only in the second period. Apparently this change in regimen did not result in alteration of the remission rate. Nevertheless one cannot exclude that the number of patients who went into remission was influenced by the tendency to operate earlier in the second period.

Parenteral nutrition in severe ulcerative colitis can provide adequate nutrition (1) and be of value in preparing the patients for the trauma of colectomy (3). It is, however, uncertain whether such treatment can induce a remission. In this material there was no change of the remission rate after introduction of parenteral nutrition. Improvement of the colitis on such treatment appears to be the exception rather than the rule (3, 4). Nevertheless Solassol et al (8) reported that remission was obtained and surgery avoided in 6 out of 12 patients receiving total intravenous nutrition without the addition of steroids.

The value of using steroids in acute severe colitis is disputed. It is possible that steroids may promote remission in severe colitis (6, 9, 10). Nevertheless the effect of corticosteroids on the fatality rate in severe attacks appeared to be less than in moderate attacks (2), and in two recent publications (6, 10) steroid treatment in severe colitis was associated with an overall mortality above 10%. A controlled trial may be necessary to show whether steroids have a place in the treatment of severe colitis.

Early surgical treatment of severe attacks has been associated with a reduced fatality rate (4, 5), and the tendency to earlier surgery in the period 1971–1975 in the present series may have contributed to the reduced mortality together with improved parenteral nutrition.

Comparing two materials composed of individuals drained from the same area but at two different periods is inferior to a controlled trial, which would be the ideal way to encircle the optimal treatment of ulcera-

The attacks of severe ulcerative colitis were classified according to the criteria of Truelove and Witts (9) which are 1) Severe diarrhea (six or more motions a day) with macroscopic blood in stools 2) Fever (mean evening temperature more than 37.5 C or a temperature of 37 8°C or more on at least two days out of four) 3) Tachycardia (mean pulse rate more than 90 per minute) 4) Anaemia (haemoglobin 75% or less — allowance made for recent transfusion) 5) E. S R. much raised (more than 30 mm in one hour)

The mean values of the pulse rate the number of stools per day and the temperature the two first days of the severe attack were calculated In the period 1966—1970 the mean number of stools was 8.9 (range 6—20) the pulse rate 105,9 (76—140) and the temperature 38.3 (37 1—39.9) In the period 1971—1975 the mean number of stools was 10.5 (6—20) the pulse rate 102,8 (84—127) and the temperature 38 1 (36,7—39.2)

Before 1st of January 1971 medical treatment of patients with severe ulcerative colitis included steroids, which were given to 19 of 30 patients. Total parenteral nutrition was not given After 1st of January 1971 closer cooperation between the departments of Medicine Surgery and Anaesthesiology was established. Patients with attacks of severe colitis were given total parenteral nutrition for five consecutive days. The intravenous regimen included proteins (2 g/kg bodyweigt/day) and total calories 30/kg bodyweight/day Corticosteroids were given to all 23 patients except one in whom perforation was suspected. Prednisone was given orally until July 1974 After this time hydrocortisone 240 mg daily was given intravenously in four divided doses. Antibiotics were not included in the regimen. Conservative treatment was continued in patients who went into remission or showed clinical improvement within the first 3—5 days. No improvement within the first few days or a deterioration in clinical condition was considered an indication for immediate colectomy The mean duration of hospital medical treatment prior to surgery in the period 1971—1975 (17 days, range 7—36 days) was reduced compared to the period 1966—1970 (31 days, range 0—106 days)

Results

The outcome of severe attacks of ulcerative colitis is shown in table III The proportion of patients who went into remission and were able to continue medical treatment was the same in the second (52%) as in the first period (50%)

Table III *Short term results in patients with severe ulcerative colitis*

Result	1966—1970 (no)	1971—1975 (no)
Remission		
(cont. med treatment)	15	12
Surgery	15	11
Deaths (only after surgery)	3	0

Early mortality (table III) In the period 1966—1970 (30 patients 33 severe attacks) three patients died after surgery whereas none died during medical therapy The early mortality in this period was 10% whereas there was no early mortality in the period from 1971—1975 (23 patients, 26 attacks)

The operative procedures in the attacks of severe colitis are shown in table IV The patient who died after colectomy and ileorectal anastomosis a 42 year old woman was operated after a 6-week period of conservative treatment of her first severe attack. She died from peritonitis due to anastomosis leakage The patient who died after colectomy and ileostomy and closure of the rectum was a 14-year old girl She was submitted for immediate operation due to perforation of the colon After the operation she was severely ill due to septicemia and died after 3 months The patient who died after proctocolectomy a 44 year old woman was operated after 2 months hospital treatment of her

Table IV *Operative procedures in severe attacks of colitis*

Method	1966—1970 (no)	1966—1970 (dead)	1971—1975 (no)	1971—1975 (dead)
Colectomy and ileorectal anastomosis	4	1	0	0
Colectomy ileostomy and proximal closure of rectum	2	1	2	0
Colectomy ileostomy and sigmoidostomy	4	0	2	0
Total proctocolectomy and ileostomy	5	1	7	0
Total	15	3	11	0

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Physiological aspects on the diagnosis of renal artery stenosis

J Ofstad and Y Willassen

Hypertensive disease caused by stenosis of the renal artery in man seems to correspond fairly well with the experimental counterpart in different laboratory animals. Increased secretion of renin into the blood stream occurs early in the pathogenesis. Comparison of the renin concentration in the renal venous blood from both kidneys (renin ratio) is at present considered to be the most important diagnostic and prognostic method in this condition. Renin concentration ratios varying from 1.5 to 2.5 have been reported as highly indicative of curable renal hypertensive disease in man. A considerable number of patients cured by operation have ratios less than these values.

Some questions should be considered before renin concentration ratios are applied in clinical practice

1) What is the error of method in renin ratio measurements?
2) What is the relation between the ratio of renin concentrations and that of renin secretion rates?
3) Does the ratio express an all -or- nothing kind of mechanism or a graded biological phenomenon? (What does the population of the ratios look like?)

Error of method in renin ratio measurements

On the right side sampling from the short main stem carries the risk of sampling only from one section of the kidney if the catheter is pushed too far into the vessel or the possibility of aspirating blood from the vena cava if the catheter has not been introduced far enough into the renal vein. On the left side the renal vein is so long that the risk of introducing the catheter into a branch is minimal. The greatest errors in sampling on the left side are caused by aspirating blood from the spermatic or ovarian vein emptying in the inferior vein wall or from the left suprarenal vein emptying in the superior vein wall.

One main cause of sampling errors on both sides is the laminar character of the blood stream in the renal veins. Although the evidence of laminar flow in the renal vein is indirect, it is quite conclusive. The Reynold's number of the blood stream is much lower than that thought critical for the onset of a turbulent flow regime When roentgen contrast medium is injected into the renal vein, the findings are well compatible with a laminar type of flow and the difficulties in obtaining flow measurements using indicator dilution techniques (dye dilution or thermodilution) is probably also partly due to the presence of this type of flow We have ourselves in experiments with dye dilution measurements of the renal blood flow in man observed that dye injected into a branch feeding only the lower pole of the kidney was not recovered from the venous flow when the sampling catheter was placed in the upper part of the renal vein. The presence of a laminar blood flow has the obvious consequence that measuring renin concentrations in renal venous blood may be of little help in conditions where only a part of the kidney

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abdominal and intrathoracic pressure, transient changes of considerable magnitude may occur. During an ordinary Valsalva maneuvre the hemoglobin concentration in the renal vein may drop 20—30 per cent (fig. 1) probably due to influx of glomerular filtrate or interstitial fluid into the capillaries (3). (This is probably also the reason why the kidney dimensions are smaller and its form less elliptic on roentgenograms taken during an arteriography with the Valsalva maneuvre than during an intravenous pyelography with ureter compression when the kidney tissue pressure, and volume, is greatly increased). This hemodilution may affect the renin measurement. The fact that the kidneys participate in the baroreceptor trigged sympathetic reflex induced by the Valsalva maneuvre is probably more important however. Even less extreme sympathetic stimulation may influence the renin secretion rate itself. From every point of view there is no substitute for physiological conditions during physiological measurements.

Autoregulation, renin secretion rate and the clinical presentation of hypertension in renal artery stenosis

Three mechanisms seem to be the most important in the control of renin secretion rate in the laboratory animal (4): the myogenic, in which a supposed dilatation of the afferent arteriole increases the renal secretion rate; the nervous in which stimulation of sympathetic nerves increases the renin secretion rate, and the macula densa feedback mechanism in which a change in composition of the tubular fluid in the macula densa region of the distal tubule influences the renin secretion rate. It can hardly be empasized enough that our knowledge of these matters in laboratory animals is for the far greater part based upon acute experiments, and that the patients we deal with in the medical wards have suffered from their condition for a long time, often for several years. How-

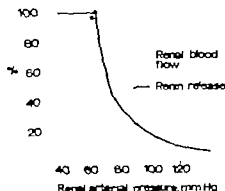


Fig. 2. Relation between renal arterial perfusion pressure and renin secretion rate.

ever, with this general reservation the present knowledge seems to be in favour of the myogenic mechanism as a dominant factor of the renin release in man. In animal experiments the macula densa mechanism is probably eliminated in the arteriostenotic kidney model with a non-filtering kidney (the endocrine kidney), a model that has also been observed in man. The denervated arteriostenotic kidney model of the laboratory animal has its clinical counterpart in the transplanted kidney with arterial stenosis and renin induced hypertension. Furthermore the probable increase of the renin secretion rate during passive head up tilting of paraplegic patients (5), in which nervous stimulation is eliminated, and with a probable shut down on the glomerular filtration during the tilting procedure, also support the idea of the myogenic mechanism as the most important for the renin secretion in man in general as well as in renal artery stenosis with hypertension during physiological conditions.

The relation between the myogenic response during renal perfusion pressure decrease and the renin secretion rate has been well described (6) (fig. 2). The renin secretion rate is correlated with the autoregulation of the renal blood flow and has a maximum value when the renal resistance has reached its minimum. During further reduction of the perfusion pressure the renin secretion rate

is affected, i e. in conditions such as renal infarction stenosis of minor arterial branches and a renin producing tumor where the renin ratio has been reported to be close to normal in some patients.

The measurment error includes the physiological variation of kidney function, the sampling error and the errors due to the chemical analysis of the sample. The last factor can easily be estimated and subtracted from the other errors mentioned The remaining error is then due to sampling and to physiological variations. For a given position of the sampling catheter in man, (on the left side 3/4 vertebral width and on the right side, 1/3 vertebral width outside the contour of the vertrebal column) the error in measuring E-PAH was found to be 0 009 when the chemical analysis error was excluded (PAH-concentrations varying from 1 to 5 mg%) (1) Thus under these circumstances the sampling error is small and of little importance in the calculation of renin concentration ratios The risk of aspirating blood from the spermatic (or ovarian) vein i e. from the lower part of the renal vein cross section is probably negligible when the performed catheter bend is such that the tip of the catheter is kept in permanent contact with the upper part of the venous wall during the sampling procedure. When in doubt it is wise to combine blood sampling for renin with simultaneous measurements of the E—PAH which is a far more sensitive check of the catheter position than the oxygen saturation

The greatest error of method in renin ratio measurements is probably the error of renin analysis which as a rule is not given in the published studies. In the best hands (2) this error is not less than 12—15% (variation coefficient) for single measurements and correspondingly greater for the renin ratio which includes two separately measured values The analysis error is probably greater when the renin concentration is low (3), this may partly explain why the prognostic value of

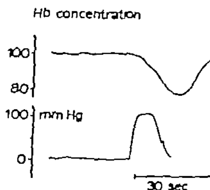


Fig 1 Hemoglobin concentration in renal venous blood (upper curve) during the Valsalva maneuvre. Pressure recording (lower curve) from the renal vein.

an elevated renin concentration ratio is greater when the renin concentrations are high than when they are at a lower level.

The question to be answered is what does a ratio limit of let us say 1 5 really mean? If the measurement error is 15% (variation coefficient) and 2 SD is chosen as an acceptable measure of reproducibility a true renin ratio value of 1 5 is in fact the middle of a population of ratios varying from 0 6 to 2 4 when the ratios are based upon single analysis of the concentrations involved Another way of looking at this ratio limit is that it might express the lower limit of a population of ratios around an unknown mean value. If so, this ratio would be 2 14 (unknown renin ratio —2 SD) The effect of varying organ function will increase the range of ratios furthermore In conclusion the stated critical renin ratios are as a rule meaningless because they lack elementary information about the error of method without which they can not be interpreted It might seem rather astonishing that such ratios are given decisive weight as to whether patients should be operated upon or not

Some early authors paid attention to the small variations of the hemoglobin concentration in renal venous blood during the blood sampling These changes can be neglected in the relaxed patients. However if the patient for one reason or another is straining and thereby increasing his intra

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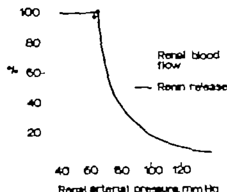


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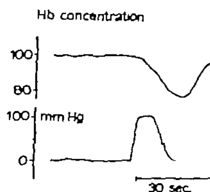


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The functional relation between renin secretion rate and renal blood flow with a curvilinear increase of the secretion rate with falling blood pressure indicates that the hypertension of renal artery stenosis may be a graded biological phenomenon with a bor derline stage overlapping the normal pressure condition. A gradual blood pressure increase over a period of time has been observed in some patients with renal artery stenosis, especially in the patients with stenosis in the artery of transplanted kidneys. Such infor mation is the exception rather than the rule.

Decisive for the clinical presentation of a status macendi of the stenotic hypertension is the time course of the stenotic process itself. The stenosis caused by atheromatosis at the aortic opening of the renal artery seen in elderly patients and at the arterial junction in transplanted kidneys is known to progress slowly and thus permit gradually increasing blood pressure to be observed clinically Less is known about the natural course of the stenosis seen in the middle part of artery commonly caused by hyperplasia of connective tissue and muscle cells in the arterial wall. The most common form, where the hyperplasia is located mainly to the middle layer of the artery has as a rule reached a stationary stage when diagnosed (9) Neither the trigging mechanism nor the negative feedback mechanism of the hyper plasia are known (The constant localization and the probable connection with primary hyperaldosteronism suggest the local physical factors as the initiating pathogenic factors. The negative feedback is probably not totally dependent on flow reduction as hyperplasia in some patient do not decrease the arterial lumen at all) Present knowledge of the

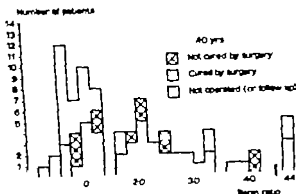


Fig 3. Renin ratios in patients with hypertension and different degrees of renal artery stenosis salt intake normal (3, 10, 11)

hyperplastic type of stenosis is compatible with the idea that this hyperplasia develops relatively rapidly and is then arrested per manently but the question whether the time of development should be counted in days or in months can only be answered by guess-work. Furthermore, the time course of the development of the hypertension is probably dependent on the progression of the stenotic process at the moment when it provokes the pressure drop This is probably a late pheno-menon in its time course. Model experiments in our laboratory indicate that the minimum diameter of a short stenotic segment must be reduced to about 30 % of the diameter proxi mal to the stenosis and that the presence of a postenotic turbulent flow type with trans-formation of pressure into kinetic energy is important in precipitating the necessary pressure drop over the stenosis. The diameter change necessary for this pressure drop to occur is probably very small. This may ex plain the difficulty reported in correlating the diameter of the stenosis with the hyper tension.

The clinical observation that the hyperten-sion in renal artery stenosis, as diagnosed with our present methods, is apparent as an all or nothing phenomenon is thus com-patible with the ideas referred to above. Another relevant question is whether or not the renin ratios in a group of patients with

is constant The implications for the measurements of renin concentration in the renal venous blood are obvious. When the blood pressure is decreased below the level of the autoregulatory range the renin concentration is a hyperbolic function of the renal blood flow At higher pressures however the renin concentration is is directly correlated to the renin secretion rate The critical question of whether the measurement of renin concentration in the renal veins is just a cumbersome method of measuring renal blood flow may thus be reduced to the question of whether or not patients with renal artery stenosis have pressure reductions below the range of autoregulation of renal vascular resistance.

When autoregulation is exhausted by lowering of the perfusion pressure in acute animal experiments, the glomerular filtration rate and the renal blood flow drop as a linear function of the perfusion pressure, the filtration fraction is reduced the socalled tissue pressure drops and the fractional absorption of sodium and water increases In patients with renal artery stenosis and renin induced hypertension lowering of the filtration fraction is usually observed The blood flow per gram of cortical tissue is reduced in some patients but not in all and in some patients only slightly so (7) Preliminary measurements of the intrarenal venous pressure indicate that the tissue pressure in these patients is reduced in the stenotic kidneys when compared to the contralateral kidneys with normal arteries (8) This might of course be due to an increase of the tissue pressure in the contralateral kidney exposed to the elevated perfusion pressure, in analogy with what Loewenstein has reported in patients with essential hypertension in mannitol diuresis This increase of the wedged pressure has, however not been present in similar patients studied in antidiuresis (8) The glomerular filtration rate is seldom reduced to very low values, i e. half that of the contralateral kidney or lower The percentual increase of sodium and water reabsorption is as a rule increased in the stenotic kidney Although the present data do not permit a statistical estimate, the observations available in patients with hypertensive disease due to renal artery stenosis incidate that the perfusion pressure has dropped slightly beyond the lower limit of the autoregulatory range in most patients.

The clinical value of renin concentration measurements in renal venous blood is therefore probably only to a minor degree influenced by the variation of the renal blood flow On the hypotensive side of autoregulation the effect is an increase in the renin concentration with decreasing blood flow potentiating the concentration difference between the kidneys and increasing the renin ratio The clinical problem is, however not the false positive concentration ratios, but the false negative ones Measurement of the renin secretion rate will therefore probably add little to the diagnostic value of the renin concentration measurements when the perfusion pressure has dropped below the area of autoregulation.

If it is permitted to reason by analogy from the acute experiments in the laboratory animal to the chronically diseased patients renin hypersecretion capable of causing hypertension may accur before the perfusion pressure has dropped below the limit of autoregulation i.e. when the blood flow and interstitial pressure are still normal and the changes in filtration fraction and sodium handling which is not autoregulated are small Measurement of the renin secretion rate will probably add little to the diagnostic value of the renin concentration measurements also in this situation as the secretion rate is directly proportional to the renin concentration. The diagnostic problem in this patient group which may also be complicated with the presence of patients with high sensitivity to angiotensin may probably be solved when the angiotensin antagonists become available for routine clinical use It would not be surprising if the level of

hypertension in this group would show to be lower than in patients with a greater drop of the perfusion pressure because of a lower renin secretion rate, and that the acid but not necessarily sensitive, diagnostic test of cure by surgery may be less useful in this patient group

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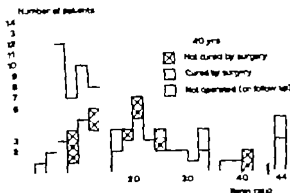


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The clinical observation that the hypertension in renal artery stenosis, as diagnosed with our present methods, is apparent as an all- or nothing phenomenon is thus compatible with the ideas referred to above. Another relevant question is whether or not the renin ratios in a group of patients with

different degrees of renal artery abnormalities are a graded biological phenomenon In figure 3 such ratios from 80 patients on a normal salt diet are presented The population of ratios seem to be biphasic with peaks at about 1 0 and 2 0 This may seem to be an additional point in favour of the interpretation of hypertension of renal artery stenosis as an all-or nothing phenomenon However quite a number of the patients with normal ratios were cured after surgery and in some patients the ratio moved from the peak concentrated around 1 0 to values above 2 0 the dietary salt intake was reduced One can speculate as to whether the hypertensive patients with normal ratios who were cured after surgery were individuals with unusually high sensitivity towards angiotensin and/or were the ones in whom the renal perfusion pressure had not yet fallen below the range of autoregulation

The usual concept of the relation between the afferent arteriolar diameter and the renin secretion rate is that the secretion rate rate increases when the arteriole dilates i e when the transmural pressure and the tangential tension decreases This follows from another concept, namely that the autoregulation takes place in the afferent arteriole There is no published observation neither of the pressure nor of the diameter of this vessel during autoregulation In the rat (12) the autoregulatory vasomotion is reported to take place mainly in the interlobular artery during autoregulation and only to a minor degree in the afferent arteriole Differences between species may be present we have with a microsphere injection technique measured the diameter of the afferent arteriole in the dog during autoregulation The diameter of the afferent arterioles increased in each of three equally thick cortial layers when the pressure was reduced The diameter increase was great enough to explain the whole decrease of the vascular flow resistance

The anatomical localization of the autoregulatory process is mainly of theore interest and is not of particular practic importance in renal artery stenosis. In othe forms of renal hypertension where lesions c the interlobular artery are a main feature o the vascular pathology (such as in Wegener granulomatosis and sclerodermia), the ans to the question of whether or not autoregul ation takes place in the interlobular may be of nosographical value. This sub is however outside the scope of this paper.

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Supplementum 602

The Marstrand Hypertension Meeting 1976

Report from the 8th Nordic Hypertension Meeting in Marstrand, Sweden, June 11–13 1976

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CONTENTS

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INTRODUCTION

I 1958 Dr Haako Storm Mathise initiated a serie of rather informal m etings b twe n hyp rtension re earch rs from the four Nordic o tries Denmark Finland Norway nd Swede These meetings were h ld altern tiv ly i a h of the fo r o untrie usu lly with a two y ar interval The purpo e of the e gathering w s to facilit te exchange of id s and to promote di c sio s of new findi gs o p rson l level

Th ks to Dr Teg Hilde this id a ha b e revit lized nd at the same time th Nordic Hyp rten i n me ting hav become open to a wider udi nce Th 1976 Nordi Hypert n ion Meeting w th eighth su h meeting since 1958 d the fi t of th modified ki d It was held t Marstrand a small ya hting ce ter north of Gothenburg Sweden in J 1976

For th first time it w s decided to publish th p pers of a Nordic Hypert i M eti g and this suppleme t to Act Medica S andinavi a i th e th first u h oll ctio of papers Th d cisio to p bli h the papers w based on two mai pri ciples Firstly it was felt th t the p pers l cted for pre ent tio at the me ting would give rather f l ar ll iew of om of the current hypert n io re rch a tivitie that are going on i the N rdi o ntries nd se ondly it was idered a well found opportu ity to coll ct i place papers that would not ordin rily app ar i th same j rn l It was h ped that this wo ld timulate li i i s nd imal workers alike to wid their fields of t re t

Finally the Org i ing Committ e wishes t e pre it si ere gratitude t Mr Per Erik Hörnkvist of ICI Ph rma AB who mad the Ma strand Meeting 1976 nd th printing of this s ppleme t possibl through ge erous gra t

Le nart Hanss o
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Sahlgren s Hospital
S 413 45 G th nburg Swede

INTRODUCTION

In 1956 Dr Haakon Storm Mathisen i itiated a seri s of rath r inf rmal me ting between hyperte sion re e rcher from th fo r Nordi c untries D nmark Fi l nd Norway nd Sweden These me tings were held lternativ ly in each of the fo r countrie us ally with a two year interval Th purpose of these gathering was to facilit te exchange of ideas and t promote di cussions of new finding n a person l l vel

Tha ks to Dr Tag Hild n this ide has be r vitalized and at the ame time the N rdic Hypertension me tings ha e b come op to wid r audien e The 1976 Nordic Hypert sion M eting was the ighth a ch me ting in a 1958 and the first of the modified kind It was held at Marstr nd small yachting nter north of Goth nburg wed n i J e 1976

For the first time it was d cided t p blish th paper f a Nordi Hyp rte sion M eti g d this s pplement to Act Medi a Scandin vica is thus the first s oh c llection f pape s The deci io to publish th p pers wa b d o two mai pri ciples Fir tly it w s felt that the papers sele ted f r pres ntation t the me ti g would giv a rather ef l over- ll view f som of the cu re t hyp rt sion re s r h a ti ities that re goi g o in th Nordic tries and se ndly it was co idered a w ll found opportu ity t c ll ct i plac pap rs th t wo ld not rdi arily appe r i the ame j rnal It w s hoped that this would timulate cli i ia d s imal wo kers like to wide their fi lds of i t r t

Fi ally the Orga izing Committe wish s t press its sin re gr titud to Mr Per Erik Hörnkvist f ICI Pharma AB wh made th Marstrand Me ti g 1976 and the printi g of this pplemе t po sibl through a g erou g a t

Le art H sso
D p tme t of Medici I
Sahlgren s Hospital
S 413 45 Gothe burg Swed

INTRODUCTION

In 1958 Dr Haako Storm Mathisen initiated series of rather informal meetings b twee hype tension research rs from th f ur Nordic co tries D nmark Finland Norway and Swede The e meetings wer held alter atively in ch of the fo r co ntri s s ally with a two year interv l The p rpo of these gatheri gs was to facilitate e cha ge f id d t promote discus io of ew findings on a perso al lev l

Tho ks t Dr Tag Hilde this idea has been revitalized and t the sam time th Nordic Hyperto sio meeti gs have b ome pen to a wider audi ce The 1976 N rdic Hypert n si M eti g wa the eighth e h meeting si e 1958 a d th first f th modified kind It was held t Marstrand a small ya hting c nter rth of G the burg Sweden in J 1976

For the fir t time it was decid d t p bli h th pap rs of N rdi Hyperten io M eting nd thi upplem t to Act Medica Scandina ica is thu the first such collection f p pe Th de i i to p blish th p p rs was b ed on two mai principl s Firstly it was felt that th p p ele ted fo pre tatio at the me ti g wo ld gi a rather e f l ov r all iew f some of the current hypertensi n r e arch ctivitie that re goi g on i the Nordic countries and econdly it w o sid red well found opport ity to ll t i n ple p pers that wo ld ot ordinarily appear i the sam j rnal It was hoped that thi would stimul te cli icia nd imal work re like t wide their fi ld of i t re t

Fi lly the Orga izing Committee wishes to e p ess it i re g titud to M Per Erik Hörnkvi t of ICI Pharma AB who mad th Marstrand M eting 1976 nd the printi g of thi pplement p ible thro gh a g ero grant

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THE SAUSAGE STRING PATTERN IN THE PIAL VESSELS IN ACUTE ANGIOTENSIN INDUCED HYPERTENSION VASOSPASM OR VASODILATATION?

Svend Strandg ard John V Jones Eric T M cKenzie David I Graham nd J Keith Farrar

From Medical Department TA Rigshospitalet Copenhagen Denmark Department of Medical Cardiology Department of N uropathology and Wellcome Surgical Rese rch Institute Univer ity of Gla gow Scotl nd and Department of Biophysics Univer ity of Western Ontario London Canada

In Byrom cla ical work from 1954 on hyperten ive e cephalopathy i the rat (2) the pial arterioles at high blood pre sure howed a pattern of alternating con trictions and dilatations which has become known as the sausage- tring or bead tring phenomenon The constricted segments were thought to represent hypert nsive vaso pasm i e of an overconstriction of the vessels leading to a critical decrease in cerebr l blood flow Gie e (6) found the same pattern in intestinal arterioles in rats in acute angiotensin induced hype tension Colloidal carbon was observed to penetrate thes vessels in the dilated but nev r in the constricted segments Byrom later revised his concepts and in 1969 (3) pr sented ew evidence in favou of forced vasodilatation a the cruci l event in the pathogenesis of acute hypertensive encephalopathy

Cerebral blood flow is normally autoregulated i e kept con ta t by n intrinsic mech nism varying the calibre of the arteriol during blood pressure changes A number of studies from

Thu in the pr sent study the sausage-string phenomenon was a transitional phenomenon between autoregulatory vasoconstriction and the generalized va odilatation caused by very high blood pressure No igns of vasospasm were found Recent evidence (1) favours the possibility that stimulation of the cervical sympathetic nerve shifts the upper limit of autoregulation of cer hral blood flow to a higher pres ure Work on the present experimental model with cervical sympathetic stimulation added is now in progress

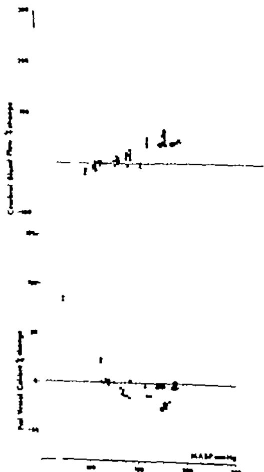


Fig 1

1971-76 have shown the existence of an upper blood pressure limit of autoregulation beyond which cerebral blood flow increases (5 9-13) This has been called the break-through of autoregulation (7) Only one study has claimed the existence of hyper tensive vasospasm in terms of acute focal flow decreases (4) The working hypothesis of the present study (6 8) then was that the constricted segments of the sausage-string pattern might not be vasospasm but the remnants of autoregulatory vasoconstriction whereas the dilated segments might be those who had given way to the high transmural pressure

The study was carried out in cats anesthetized with chloralose and maintained at normocapnia (arterial PCO_2 about 32 mmHg) by means of a respirator The surface of the brain was exposed by a unilateral parietal craniotomy and covered with mineral oil Vessel calibre was measured by the image-splitting technique In the parietal cortex in the opposite hemisphere platinum electrodes were inserted for measuring cortical blood flow by the clearance of inhaled hydrogen Blood pressure was increased gradually and stepwise by the intravenous infusion of angiotensin II

Fig 1 shows the results obtained from 5 cats where measurements were obtained throughout the study in segments of vessels that eventually developed into the narrow parts of the sausage-string phenomenon Resting pressure in the cats was 9o-12o mmHg the latter taken as reference pressure in the graph With induced hypertension sausage-string appeared regularly in vessels with a resting diameter of loo q or less when the mean pressure reached about 17o mmHg At higher pressures a generalized vasodilatation developed In the graph encircled points represent measurements made after the development of sausage-string It is seen that no further constriction took place and that the flow was in fact increased when the constriction-dilatation pattern appeared A postmortem neuropathological examination revealed no signs of ischemic cell changes not related to the craniotomy or electrode insertion

INCREASED VASCULAR PERMEABILITY FOR PLASMA COMPONENTS

DURING ACUTE ANGIOTENSIN HYPER^TENSION

Fi Ol

University of Copenhagen Institue of Hygien Copenhagen Denmark

Th xpe im t 1 t h iqu R t w ighing 1 0 160 g m w ana sth ti d complet ly A th t r of poly thyl n w pl d i th f m al v in a d giot in was inj ct d int av ly v ry fifth min t duri g 3 4 h ur in a do of ½ 1 mi og n per i je ti With thi p du th blood p u will i f om th n rmal 1 v 1 (ab t 90 100 mm Hg) t bout 160 mm Hg ft very gi t in i j tion and n m 11 b fo e th t angi t in i j i Wh th xp im t 1 p i d w fini h d th im 1 w kill d with 1 rg d of b bitu t (anyth 1 ⊗) i t pe it ally d diff t o g n w e fi d i fo alin fo t dy d th fluo at mi r op As t m f th a tiv plasm compon t at rum lab 11 d with th fluo h om Li mi Rhod mi w d (1) Th fl ch ome 1 b 11 d rum w ircul ting i 11 of th p i ment 1 p i d

R lt

Fl t mi o opy Th mi d o g w follow Th h t th kidn y d th ta

Th h t Th perm bility of the fl or sce t rum p t i into the w 11 f th on ry t i w p r d found i ly on ry t ry The i d p m ability was f 1 d took pl i t 11 th thi k of th 1 Cont ry to th ry t i m 11 t i d t iol

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INCREASED VASCULAR PERMEABILITY FOR PLASMA COMPONENTS

DURING ACUTE ANGIOTENSIN HYPER EUSION

Fl n Ol

University of Copenhagen Institu of Hygien Copenhagen Denmark

Th xp im t l t h iq R t w ighi g 150 180 g an w an th ti d mpletely A th t r of poly thyl w pl d in th f m l v i nd gi t n in w s inj ct d int a ously ry fifth minut d ing 3 4 hou in a d of ½ l ni og am p r inj tio With thi p du th blood p will i e f om th ormal l v l (ab ut 90 100 mm Hg) t b t 160 mm Hg ft r very a giot in i j ti d n rm li b f e th t giote sin inj n Wh th xp ime t l pe iod w fini h d th a im l w kill d with l rg do of b rbitur t (mythal ®) i t a pe ito lly and diff r t g we e fix d i form lin fo t dy d th flu ce t mi ro ope As t f th n ti e pl m mp t t m lab ll d with th fluo h ome Li min Rhodami w us d (1) Th fl chrom lab ll d erum w ircelati g in ll of th pe i me t l p i d

Re lt

Fl t mic opy Th min d o g w e follo Th h rt th kidn y d th t

Th h t Th p rm bility of th fl t m prot i i t th w ll of th o on ry rt i w p d found i ly on on ry t ry Th i d p m bility was foc l d took pl c i to ll th thi k of th v s l C t ry to th co n ry t i m ll t ri d t iol

in the myocardium sho ed a very marked penetration and deposition of fluorescent proteins in the wall This deposition was observed both in the tunica intima and in the tunica media and in all tho circumference of the vessels

The kidney The permeability was sparse and focal in the large arteries and especially localized to branching areas where it took place into all the layers of the arteries Contrary it was recognized that the permeability of small arteries and arterioles in the cortex and the medulla was very marked followed by a deposition of fluorescent proteins in the whole thickness of the walls and usually in the whole circumference of the arterial vessels

The aorta: Two types of permeability for plasma components seemed to take place into the aortic wall One of the types was localised to the branching areas In these areas the penetration took place into all the layers of the aortic wall and could be observed in the tunica intima in the tunica media and partly in the tunica adventitia In all other parts of the aorta the penetration took place mainly into the tunica intima and the most luminal part of the tunica media This increased penetration and d position of the fluorescent proteins in the tunica intima and the most luminal part of the tunica media seemed to be rather diffuse than focal

As a preliminary re ult it was found in one rat which was given the above mentioned experimental period during four consecutive days that the aortic wall had reacted morpholo gically on the hypertensive damage in a way which seemed to be the initial stage of the developmen of athero clo ci

Ref

1) Ol en F Pe tr tion of ir ulating flu r ec t p ot in into w ll of rt riol and v nule in ts with i t rmittent a ute angi ten in hype t n i Act p th mi robi l S andin v 74 325 332 1968

Ultrastructural arteriolar lesions and permeability changes in acute angiotensin-induced hypertension

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Anatomy Department C University of Copenhagen
1 Universitetsparken 2100 Copenhagen Ø Denmark

The small intestine from Wistar Furth rats with angiotensin-induced hypertension for the duration of 1 hour was fixed in vivo by simultaneous injection into the intestinal lumen of phosphate-buffered 6 p c glutaraldehyde and flushing of the abdominal cavity with the fixative for 5 minutes Then the intestine was taken out and immersed in the same fixative for 18 hours FITC-dextran (Pharmacia AB Uppsala Sweden) was injected intravenously 20 minutes prior to fixation in preparation for permeability studies The FITC-dextrans were visible in vivo and following the fixation by use of a fluorescence-stereomicroscope Following dehydration and embedding in Epon the FITC-dextrans in tissue sections were made visible by fluorescence microscopy and by electron microscopy using a special methodology which is to be published

Results: Focal lesions of the arteriolar wall were found in dilated parts of the submucosal intestinal arterioles (Fig 1) (Giese 1973 Thorball & Olsen 1974) The most severe lesions were always found in relation to endothelial gaps and consisted in necrosis of the media and blood-/plasma-insudation into the arteriolar wall The slightest changes consisted of widening of the subendothelial space changes in the structure and thickness of the internal elastic lamina and also irregularity of and occurrence in the smooth muscle cells of large vacuoles aggregation fragmentation and waste of the filaments and change of the electron density of cytoplasm The wall in between the focal lesions in the dilated parts of the arterioles was stretched and thin (Fig 1) In the contracted parts of the arterioles (Fig 1) no pathological changes were found

In the angiotensin-treated rats tracer particles (FITC-dextran MW 40 000) were found in the lumen in endothelial vesicles in the subendothelial basement membrane in the internal elastic lamina in

intercellular space in the media in the adventitial basement membrane and in the adventitia in as well dilated as contracted arteriolar segments (Fig 2) Preliminary results showed that in normotensive control rats the amount of tracer particles in the endothelial ve icles in the subendothelium and in the internal elastic lamina was less than in hypertensive animals (Fig 3) In addition dextran particles were found in the hypertensive animals in large amounts in the focal areas of plasma-insudation and in vacuole-like structures in the smooth muscle cells in areas with slight changes

The results indicate that in the angiotensin-induced hypertension there is an overall increased permeability of the arteriolar ndothelium and a spreading of the permeated elements in the arteriolar w ll and in the surrounding tissue The interrelation between the increased permeability and the ultrastructural lesions in the development of the hype tensive vascular lesions has not been clarified

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Legends for figures:

g: glycogen j: endothelial junction E: endothelium IE: internal elastic lamina L: lumen M: Media

Fig 1: Part of a submucosal arteriole from a rat with angiotensin-induced hypertension Note the contracted part at right in the picture and the dilated part with a very thin media at left Locations of focal lesions are indicated by an asterisk x 1100

Fig 2 & 3: The intima of an arteriole from an angiotensintreated and from a normotensive rat respectively Arrows point to some of the dextran particles made visible by lead-contrasting A difference of dimensions and of electron density of the tracer particles is found There is an uneven distribution of tracer particles in the two pictures One endothelial vesicle is marked by a demarkation of small rings x 156000

THE IMPORTANCE OF THYMUS ON THE DEGREE OF INCREASED BLOOD PRESSURE AND VASCULAR DISEASE IN MICE WITH DOCA AND SALT HYPERTENSION

Ulrik Gern r Svendsen

Th University Institute for Experimental Medicine Nørre Alle 71
DK 2100 Copenhagen Ø Denmark

Hypertensive vascular di ease is characterized by thickening and degeneration of intima hyperplasia and in more pronounced case fibrinoid necrosis of both intima and media and adventitial cellular infiltration The morphology of the infiltrates are similar to the morphology of the cellular reactions in immune reactions of the delayed type for which thymus and the thymus-derived lymphocytes are necessary The cause of the perivascular inflammatory cellular reaction can be a secondary reaction to the vascular damage without pathogenic importance Studies in rats (1) with acute angiotensin II hypertension have given evidence that the cellular infiltration observed around damaged mesenterial arterioles are caused by an immune reaction of the delayed type The same conclusion was reached in experiments with transfer to normal syngeneic recipients of the acic duct cells from rats with Goldblatt 2 kidney hypertension (4) in which it was pos ible to transfer a changed cellular reactivity against damaged arterioles by means of washed thoracic duct cells with a high percentage of thymus-derived lymphocytes

The aim of the present investigation has been to investigate the importance of thymus-dependent immune reactions of the delayed type for the hypertension and the hypertensive vascular disease in mice with hypertension due to treatment with DOCA and 1% saline as drinking water (3) For this purpose nude mice are suitable as they lack recogni able thymus tissue and seem to be depleted of thymus-derived lymphocytes by both functional and hi tological studie (2) Such nude mice and their normal haired littermates of the NMRI train (Gl Bomholtgård Ltd Ry Denmark) were used Moreover nude mice which were reconstituted immunologic lly by transplantation subcutaneously with thymus from newborn mice of the same strain were used Following thi treatment the mice were able to perform immune



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reactions of the delayed type as skin allograft rejection Nude haired and nude thymus transplanted mice were unilaterally nephrectomized Commencing one week later they received 1% saline as drinking water and 6 mg DOCA (percorten[R], microcrystalline Ciba-Geigy) subcutaneously every week Untreated animals served as controls Blood pressure was recorded for one hour in the conscious semi-restrained animals after placing a catheter in the left carotid artery Fig I shows the mean blood pressures obtained in randomly selected groups of mice after 21 57 and 78 days of treatment Nude and haired mice develop similar and significant hypertension after

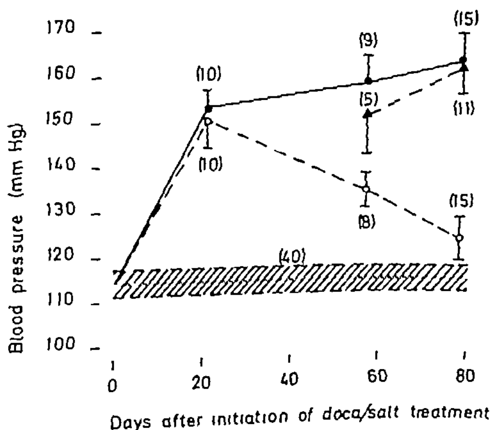


Fig I shows the mean blood pressure ± SEM in haired (●—●) nude (O—O) and nude thymus transplanted (▲—▲) mice treated with DOCA and salt The scratched area indicates the mean blood pressure in nude and haired control mice ± SEM. A significant (p < 0 001) increase in blood pressure was observed in all groups of DOCA/salt treated mice except in nude mice after 78 days of treatment (p 0 05) After 78 days of treatment a significant (p < 0 001) difference was observed between both haired and nude thymus transplanted DOCA and salt-treated mice as compared with the similarly treated nude mice Number in brackets: number of mice

21 days While haired mice are still hypertensive after 57 and 78 days nude mice have a significantly lower blood pressure which fter 78 days is nearly normal Nude mice immunologically reconstituted by thymus transplantation obtain moreover the ability to maintain a high blood pressure as haired mice after 57 and 78 days Microscopic investigations show a marked degree of round cell infiltration around intrarenal vessels commencing around the afferent arterioles and the interlobular arterie after 57 days and degenerative changes including wedge- haped infarcts in the kidneys of the haired and th nude thymus transplanted mice while no such changes were found in the non transplanted nude mice The present investigation has provided evidence for the existence of an initial thymus independent and a chronic thymus dependent phase of DOCA and salt hypertension in mice As far as the blood pressure is concerned imil r re ults as thos obtained in the present tudy have been obtained in another strain of nude and h ired mice (c57/bl/6j/Bom) with their kidneys partly infarcted but in this latter strain only few intrarenal round cell infiltrations were found So it remains an unsolved problem whether the secondary blood pressure fall observed in nude athymic mice is a direct con equence of the lack of perivascular cellular immune reaction or caused by other defects in this strain of mice

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WATER PERMABILITY IN THE HUMAN FOREARM IN ESSENTIAL AND INDUCED HYPERTENSION

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Reduction in plasma volume in hypertensive subjects with an unchanged or slightly increased interstitial fluid volume can be explained by a slight increase in venous tone in arterial hypertension (1) but other hemodynamic factors might be equally important (2) An unexplored possibility for the disparities in fluid volume regulation could be that capillarary water permability in the exchange vessels was increased in essential hypertensives Filtrative water exchange is determined by the prevailing hydrostatic and osmotic pressures the area of exchange and the hydrodynamic conductivity of the capillary memb ane The present study deals with the membrane characteristic in the ex change vessels on the forearm of essential hypertensives The capillary filtration coefficient measured plethysmographically (3) on the normal human forearm was compared to the filtration coefficient measured in patients with essential hypertension A linear correlation between mean artery blood pressure and capillary filtration from water during venous stasis was found and with an increase in mean pressure on about 4o mm mercury the capillary fil tration coefficient was increased by a factor of 2

Estimation of the capillary filtrative capacity by a hyperoncholic transient technique (4) in normal and hypertensive subjects revealed that the water permability (hydraulic condu tivity) wa unchanged in es ential hypertensives He e the are vailable for wate filtration mu t be great r in pati nt with art r al hypertension wh n exposed to veno sta is

Mea. ement of th c p llary filtr tion coeffici nt on the fo e rm of o m l befo e and du ing ang tensin II inf sion showed the ame tende cy to increase in capillary wate filtr tio with the mean a tery blood p essure as ob er ved in hyperte ve s bject The reported increase in capill y f ltr t on to wate is therefore not explainable by a hype to i m o ngiopathy Measurement of capillary filtration n the forearm during g vitatory changes in the a te i l blood pressure (elevation and depend y of the forearm el ted to h art l vel) in normals howed th ame tendency to inc es in capill ry water filtra- i with inc ease in mean arterial blood pres ure V ou pres ure inc e (a 40 mm Hg) gives a reduction i hange a ea by a veno-a teriol r reflex (5) Thi re fle might be partially impai ed when th resistance vessels in hypo ten ion has an i c e ed vascular tone Thereby tl available fo wat r ex hange du ing venous t sis w ll emai high than in normoten ive state Thi v scu l dysfunction could be a part of the explanation for the reduced plasma volume found in ambulatory patients with esse tial hyperten io

Litterature

1 Ibsen H Leth A Plasma volume and extravascular fluid volume in essential hypertension Acta Med scand 194 86 - 93 1973

2 Tarazi R C : Hemodynamic role of extracellular fluid in hypertension Circ Res 38 suppl II 73 - 83 1976

3 Poulsen H L Nielsen S L : Water filtration of the forearm in short- and long-term diabetes mellitus Diabetologia in press

4 Palm T Nielsen S L Larsen M A : Measurement of capillary water filtration in a skeletal muscle in man by an osmotic transient method Circ Res submitted

5 Gaskell P Burton A C Local postural vasomotor reflexes arising from the limb veins Circ Res 1 27 - 39 1953

High Blood Pressure as a Risk Factor for Cardiovascular Disease and Risk Factors for Hypertension

From the Glostrup Population Studies

Leif Hagerup Marianne Schroll and Hans Ibsen

In a study of fifty year old men and women examined in 1964 the prevalence and distribution of the so-called risk factors for ischaemic heart disease was obtained The same population was reinvestigated 10 years later with the same examination program This presentation describes the prevalence and incidence of high blood pressure and the risk factors for hypertension

Methods

A detailed describtion of the methods used has been published earlier (1)

The population studies in Glostrup in 1964 and 1974 are planned as a prospective epidemiological surve of a total population of 975 men and women born in 1914 At the age of fifty 802 persons were investigated and 666 at the age of sixty 627 persons were examined at both occasions

The examination program included questionnairies physical examination chest X ray ECG at est and du ing exercise and blood samples according to criteria of WHO

The blood pressure was measured after 10 minutes at rest in the supine positio with a standard cuff and a mercury manometer The diastolic blood pressure was measured at the disappeara ce of the Korotkoff sounds (phase 5)

Res lts

The incidence of cardiovascula disease and the total mortality of the populatio corresponds to the average for sex and age in the entire Danish population 57 men and 19 women out of the invited 975 i 1964 died during the following ten year period 35 men and 5 women experienced a myocardial infarction

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Results

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Litteratur

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High Blood Pressure as a Risk Factor for Cardiovascular Disease and Risk Factors for Hypertension

From the Glostrup Population Studies

Leif Hagerup Marianne Schroll and Hans Ibsen

In a study of fifty year old men and women examined in 1964 the prevalence and distribution of the so-called risk factors for ischaemic heart disease was obtained The same population was reinvestigated 10 years later with the same examination program This presentation describes the prevalence and incidence of high blood pressure and the risk factors for hypertension

Methods

A detailed describtion of the methods used has been published earlier (1)

The population studies in Glostrup in 1964 and 1974 are planned as a prospective epidemiological survey of a total population of 975 men and women born in 1914 At the age of fifty 802 persons were investigated and 666 at the age of sixty 627 persons were examined at both occasions

The examination program included questionnairies physical examination chest X ray ECG at rest and during exercise and blood samples according to criteria of WHO

The blood pressure was measured after 10 minutes at rest in the supine position with a standard cuff and a mercury manometer The diastolic blood pressure was measured at the disappearence of the Korotkoff sounds (phase 5)

Results

The incidence of cardiovascular disease and the total mortality of the population corresponds to the average for sex and age in the entire Danish population 57 en and 19 women out of the invited 975 in 1964 died during the following ten year period 35 men and 5 women experienced a myocardial infarction

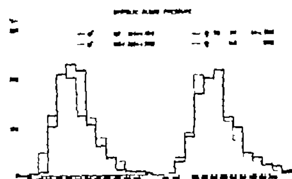


fig 1

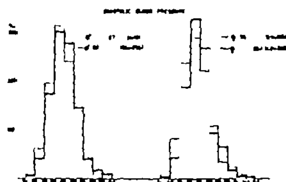


fig 2

It appears from fig 1 and 2 that the blood pressure distri bution curves in the 60 year old population are practically unchanged compared to the distribution in the same population at age 50 It has often been maintained that blood pressure increases with increasing age however this is not the case between the years of 50 and 60 At the age of fifty the systolic and diastolic blood pressures were significantly higher in the women but measurements performed 10 years later did not show any difference between men and women
The incidence of cardiovascular disease related to diastolic blood pressure is illustrated in fig 3

	MEN			WOMEN		
Diastolic blood pressure	→89	90 99	100→	→89	90 99	100→
Ischaemic heart diseases	12	9	12	2	1	1
Cerebrovascular diseases	4	4	12	5	7	16
	16=7%	13 11%	24 27%	7 3%	8 8%	17 26%
Other/or no disease	211	106	66	201	86	47
Total	227	119	90	208	94	64

fig 3

The cut off points 90 and 100 mm Hg are tentative limits between normotension borderline hypertension and hypertension The figures comprises fatal and nonfatal cases The incidence of cardiovascular diseases are clearly related to increasing diastolic blood pressure While men mostly suffer from ischaemic heart disease women are to a greater extent afflicted by cerebrovascular diseases

If we define hypertension as blood pressure higher than 155 mm Hg systolic and 95 mm Hg diastolic 14% of the 50 year old men and 10% of the women will be classified as hypertensives For the sixty year old population the figures will be the same

In the following correlations the blood pressure has been used as the independent variable to define risk factors for hypertension

The relationship of several variables to blood pressure in the base line (cross sectional) study of the 50 year olds is desc ibed in (1) A strong correlation was found between blood pressure and relative weight fasting blood glucose resting hea t rate and serum-triglyceride At the survey of 60 year olds blood pressure was positively correlated to weight glucose tolerance resting heart rate serum uric acid and to alcohol consumption In both studies a negative correla tion was found to cigaretconsumption The blood pressure in the group of smokers was on the average 10 mm Hg lower than in the group of ons okers No relationship was found between blood p essure and salt or coffee consumption or physical activity either at wo k or during leisure time No correla tion was found between se um cholesterol and blood pressure Taken together the two studies of the same population form a longitudinal survey Prospectively base line measurements of blood press re at the age of fifty is used as a dependent ariable It is strongly correlated to the blood pressure measured at the follow up used as the independent variable From fig 1 and 2 is seen that the group of hypertensives at the age of fifty still are hypertensive at the age of sixty This is true also when the blood pressures are compared in single individuals It might be expected that the borderline hypertensi es at age fifty had become hypertensive at 60

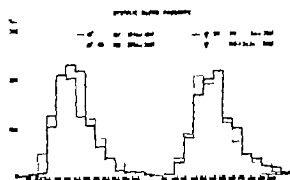


fig 1



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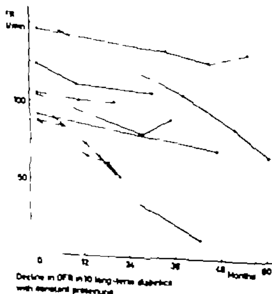
fig 3

HIGH BLOOD PRESSURE AS A FACTOR IN THE PROGRESSION OF DIABETIC NEPHROPATHY

Carl Erik Mogensen M D   Second University Clinic of Internal Medicine Kommunehospitalet 8000 Århus C Denmark

The rate of progression of nephropathy was studied in 10 young male diabetic with constant proteinuria by measuring glomerular filtration rate (GFR) ren l plasma flow (RPF) and urinary albumin excretion by exact techniques (2 3) The patients were studied for a mean period of 33 6 months with renal function te ts 2 4 times In all patients a declin in kidney function was noted the mean fall rate for GFR being 0 91 ml/min/months +/ 0 68 (SD) (figure 1) and fo RPF 4 38 ml/min/months +/- 3 23 (SD) Figure 2 shows the f 11 in GFR a a function of the level of GFR during the progre sion of n phropathy the level of GFR being the mean of two measurements from which the fall rate is calcul ted There wa a negative corr lation (r = - 0 59 2 p< 0 05) the progr ssion being more r pid t low GFR level   In 7 of the 10 subjects albumin e cretion increa sed

There was a positive correlation between fall rate of



Decline in GFR in 10 long-term diabetics with constant proteinuria

Figur 1

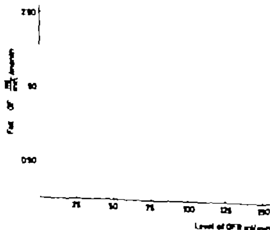


Figure 2

However this was not verified Age is probably more consistently correlated to blood pressure in younger age groups as found in other studies

Prospectively also resting pulse rate relative weight fasting blood glucose and to a smaller extent serum triglyceride was correlated to the blood pressure at the follow up

The bivariate statistical analyses performed are not able to differentiate between directly and indirectly correlated variables Possibly only some of the variables correlated to blood pressure are independent risk factors for hypertension

Multivariate analyses have been applied to data from similar epidemiological surveys in Chicago (with higher numbers of participants) (2) It is concluded from these multivariate analyses that base line blood pressure relative weight resting heart rate glucose tolerance and serum uric acid were independent risk factors for hypertension As in our study no positive association emerged between cigarette smoking and blood pressure Serum cholesterol was only significantly related in some of the analyses

Summary In a prevalence study of 436 men and 366 women 50 years of age hypertension (defined as BP 155/95 mm Hg) was found in 10% of the men and in 14% of the women The survivors were reexamined at the age of 60 The study failed to demonstrate increasing blood pressure with increasing age High blood pressure was found to imply increased risk for infarction among men and stroke among women

Blood pressure at the age of sixty showed positive correlations to base line blood pressure relative weight resting pulse rate and glucose tolerance which probably are independent risk factors for hypertension

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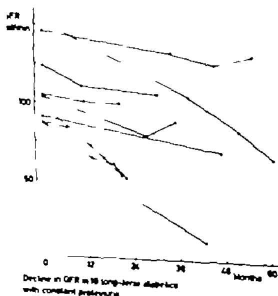


Figure 1

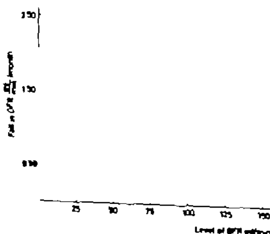
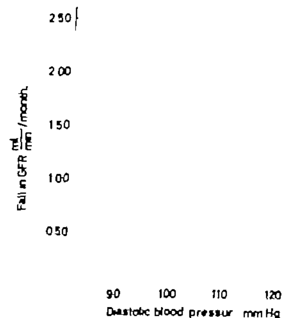


Figure 2



Fall in GFR ($\frac{ml}{min}$/month) plotted against diastolic blood pressure (at the end of the control period)

Figure 3

GFR and pre-treatment diastolic blood pressure studied in 7 patients (Fig 3) (4) Six of the patients (aged 27-35 years) were followed for 19 months during antihypertensive treatment with Propranolol alone or combined with Hydralazine and Furosemide Mean arterial blood pressure was reduced significently during the treatment period from 120 8 mm Hg +/- 12 9 (SD) to 113 7 mm Hg +/- 11 9 (SD) (2 p < 0 05) After 10 days of treatment there was a significent fall in albumin excretion from 4 09 mg/min +/- 2 58 (SD) to 3 24 mg/min +/- 2 17 (SD) (2 p < 0 02) with no change in GFR and RPF During the 19 months the fall rate of GFR calculated by regression analysis was reduced significantly as shown on table I (2 p < 0 01)

Table I

Patient	Month of observation		Number of test		Fall rate of GFR ml/min month	
	A	B	A	B	A	B
1	22	23	4	9	1 55	0 05
2	21	16	4	7	2 12	1 20
3	22	20	3	7	1 72	0 58
4	20	20	3	7	0 25	-0 08
5	30	21	3	7	0 55	-0 06
6	54	16	4	6	0 32	-0 33
Mean	28	19	3 5	7 2	1 085	0 227
SD	13 2	2 8			0 807	0 564

There was also a strong tendency to reduction of fall rate of RPF and to reduction in increase rate of albumin excretion

Table II shows that the actual GFR levels after 19 months of treatment are significantly higher than the predicted GFR-values c lculated on the ba is of liniarity of fall rate without tre tment (2 p < 0 02)

Table II

Predicted and actual GFR value after treatment

P ti nt	P treatment GFR (ml/min)	Treatment period (month)	Predi ted GFR ml/min	Actual GFR ml/min
1	52	23	16	52
2	54	16	20	32
3	67	20	33	53
4	100	20	95	102
5	106	21	94	98
6	132	16	127	138
Mean ± SD	85 2 ± 32 4	19 ± 2 8	64 1 ± 47 0	79 2 ± 40 0 (2 p < 0 02)

The predicted GFR values should be co sid red a maximal al s the progr ssion being a celerated with more pronounced n phropathy (fig 2)

The re ult trongly s ggest that high blood pressu e cha ct izing patient with diabetic nephropathy (1) is of importance fo the apid progression seen in some patients Antihy perten iv treatment may po tpone tate of renal insufficiency i such pati nt Diabetics during betablocking treatment should be controlled very car fully du to episod of hypoglycaemia occuring in some patients (5) Large trial hould be c rried out defi ing the optimal scheme of treatment

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Uses of propranolol (Letter to the Editor) N Engl J Med
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POSTOPERATIVE FOLLOW UP OF HYPERTENSIVE PATIENTS TREATED FOR UNILATERAL RENOVASCULAR OR RENAL DISEASES

A. McNai (Medi al Depa tm nt B Gl strup Ho pital) P A Gamm l-gaa d F Mathi n (Surgical D partm nt H G ntoft H pital) P FArup (Univ rsity In tit to of Pathology) M Damkjer Ni l n J Gi H Ib n A H Kapp lgaard J O Lund O Munck K H T n (Depa tm nt f Clini l Phy iol gy Glo trup H pital) C n hag n Denmark

Introdu tion

Th l ti n of hyp rt n iv pati t f u gical tr atm nt is ba ed po v ral inv tigati n In no ingl di gno tic t st pro id a l r- t progn ti tat m nt with re pect t th out-me f u g ry

In rec nt y ars nal v n us th t ri ati n with nin as sys n amples f m h of th two r nal v in and f r n mpl ha b n u ed t iv ly a part f th diagno ti work up of pa i t nd ni ion f r nal or r novas ular hypert n i n Th p po f th p nt tudy ha b n t evaluat th eff t f u gi al t atm nt and to timat th predi tiv valu f al enou athet i ti n and th preop rativ tudi

Pati nt and method

I 44 hype t n iv pati nt p rated for unilat ral r nal r no a l di nal venou ath t i ation had b n p r-f rmed pr p ativ ly Fiv pati nt ould n t be re ched r w re nabl t pa ti ipat in the follow-up mination Out f th rmai i g 39 pati nt 25 w r mal (mean g 5 y ars range 36 73 y rs) and 14 femal (m n ag 46 rang 24 64 y ars)
Th p eoperativ du ti n of hyp t nsion wa les than 6 months i 8 p ti nt 1/ 2 yea in 21 p ti t and mo than 2 y ar i l n tient Th d gr f hyp rt n i n wo ld b haract ri ed mod rat t v 31 patient h d X ray v rifi d rdiac nl gement nd/ r l troc di graphi ign of l ft v ntri ular hype trophy 21 pati nt h w d r tinal hange grad III to IV (K ith Wag n & Ba k al) T tal ren l fun tion w mod rat -ly dec ed in 9 pati nt Prio t peration 16 pati nt had ff d brova la a ident
Th p eop rativ aminati n i clud d i t av no urography i t pe enography nal angiography measu em nt of pla ma r nin

References

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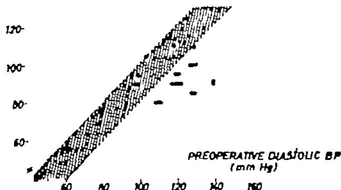


Fig 1

30 out f 35 patient (86%) b am normot iv o improv d wh r 5 patient did t h w ignifi nt improvem nt In fig 1 th blood pr ur v lu f r th la t named pati nt ar d pi ted within th hatch d r a in a rdan with th rit ria outlined bov Tw f th pati nt had unilat ral r nal art ry t n i two patient had unilat ral r nal art ry lu i n and n pati nt had unilat ral ntra ted kidney In all as unil t ral n ph - tomy had be n perf rmed

A pronoun ed r gres i n f hyp rtensiv rgan l i n w t d at th f ll w-up aminati n N patient xhibited ever r tinal hang Cardia nl rgement r hypertrophy wa pr nt in nly two pati nt ppo d to 31 patient b f r th op rati n During th f ll w-up p riod r brova ul ident oc u d in 7 pa tient All but n f th pati nt had uff red imilar att k pri to th p rati n

concentration in peripheral blood and renal venous catheterisation 35 patients had renovascular disease (22 cases of renal artery stenosis 13 cases of unilateral renal artery occlusion) 4 patients had unilateral renal parenchymal disease
The final decision in favour of surgical intervention was made on the basis of a careful integration of all available clinical and laboratory data Due attention was paid to information on the effect of antihypertensive drug treatment in the individual patient Unilateral nephrectomy was carried out in 32 patients wh r a reconstructive renovascular surgery was performed in 7 case including 2 autotransplantation procedures
The average period of observation fr m the time of operation to the follow-up examination was 32 months (range 8-60 month) The criteria laid down for asses ment of the res lt of surgical therapy were as follows:

I CURED: Normal blood pres ure according to Master s criteria without antihypertensive medication or during low do age diuretic therapy

II IMPROVED: Decrease of diastolic blood pre ure by 20 mmHg or more in patients on antihypertensive medication in a low r dosage than required preoperatively together with regre ion of hypertensive stigmata

III NOT SIGNIFICANTLY IMPROVED: Criteria for cure or improvement not fulfilled

Results:
In 4 patients the postoperative urvival period wa le than 2 months precluding a proper evaluation A priori 3 of these patients were classified as high risk patients One patient died unexpectedly 6 days after the operation from a cerebrovascular attack
For the assessment of blood pre sure change measurem nt obtain d under comparable condition were utilized The po toperative blood pressure values in patients who did not receive antihypertensive medication were compared to blood pre sure value obtained prior to preoperative antihypertensive drug treatment The postoperative values obtained in patients still receiving antihyperten ive dr g therapy were evaluated in an analogou manner

CONN S SYNDROME

A FOLLOW UP OF THIRTEEN SURGICALLY TREATED CASES

J O Lund M Dankjær Nielsen & P A Gammelgaard
(Department of Clinical Physiology Glostrup Hospital
and Urologic Department II Gentofte Hospital)

Primary aldosteronism (Conn s syndrome) due to an adrenal adenoma causes arterial hypertension and varying degrees of hypokalemia During th period 1969 1975 this syndrome was diagnosed in 13 patients who subsequently underwent adrenalectomy or extirpation of an adrenal adenoma In 1974 and 1975 a follow-up study was performed

Patient and preoperative investigations

Thirteen patients 10 females and 3 males aged 11-57 years (mean 43 years) h d art rial hypertension and hypokalemia The range of blood pressure (BP) was systolic 160-210 mm Hg and diastolic 110 130 mm Hg without any medication The mean BP (dia tolic BP + 1/3 of the pulse amplitude) was 128 150 mm Hg (mean 140 mm Hg) Three patients were initially admitted bec use of acute paralysis of the extremities In four cas s paresthesia was prominent A history of polyuria or nocturia was present in six case and seven patients were complaining of headaches In most case the hypertension and hypokalemia were di covered at routine examination during ad mis ion for other disease without any relation to adrenal disorders

Serum potassium concentration was 1 7 3 4 mEq/l In one case the hypok lemia w s p esent only intermittently There was no co relation between the degree of hypokalemia and severity of symptoms Plasma renin concentr tion wa 1 11 µGU/ml (micro-Goldbl tt units) normal r nge 6 60 µGU/ml and the excretion of t trahydroaldosterone 33-288 µg/24 h normal range 11-59 µg/24 h during inge tion of a diet with 110 mEq sodium per 24 hours During administration of exogenous mineralocorticoid (fludrocortisone 1 2 mg per 24 hours in four divided doses) the aldo terone production appeared autonomous The preoperative diagno i of an aldo terone-producing adenoma wa ba ed on the

EVALUATION OF PREDICTIVE CRITERIA (31 PATIENTS)



fig 2

UROGRAM

RADIOISOTOPE RENOGRAM

RENAL VENOUS RENIN

NORMOTENSIVE OR IMPROVED

NOT SIGNIFICANTLY IMPROV

In fig 2, the outcome of surge
the preoperative examinations
venous pyelography isotope ren
risation were available for anal
used to signify the demonstratio
The figure shows that 16 out of
in each of the three types of pr
It is worthy of note that 4 out
normality disclosed by intravenou
renography but without evidence c
tion were improved or cured 6 pa
radioisotope renography and renal
mal intravenou pyelography All t
pr ved
Out of 35 patients included in th
lateralisation of renin secretion
concentration in peripheral blood
cured or improved The results of s
defined group of patients pre entin
renin secretion were significantly
3 patient showing normal renin para
$p < 0.05$)

Concl sion:
A succesful outc me of surgical thera
of this serie of hypertensive patien
early postoperative mortality was abo
figures are encouraging Surgical the
is certainly worth-while but very car
is nece sary

CONN S SYNDROME

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EVALUATION OF PREDICTIVE CRITERIA (31 PATIENTS)

☐ LATERALIZATION

Fig 2

UROGRAM	☐	☐	☐
RADIOISOTOPE RENOGRAM	☐	☐	☐
RENAL VENOUS RENIN	☐	☐	☐
NORMOTENSIVE OR IMPROVED	16	4	6
NOT SIGNIFICANTLY IMPROVED	3	2	0

In fig 2 the outcome of surgery is related to the results of the preoperative examinations In 31 patients re ults of intravenous pyelography isotope renography and renal venous catheterisation were available for analysis The term lateralization i used to signify the demonstration of unilateral abnormality The figure shows that 16 out of 19 patient showing lateralization in each of the three types of preoperative examination were cured It is worthy of note that 4 out of 6 patients with unilate al abnormality disclosed by intravenous pyelography and radioi otope renography but without evidence of lateralization of renin se r tion were improved or cured 6 patient showed lateralization on radioisotope renography and renal venou catheteri ation but normal intravenous pyelography All the e patients were cured or improved

Out of 35 patients included in the follow-up study 32 howed lateralization of renin secretion and/or in rea ed pla ma renin concentration in peripheral blood 29 of the e patient (90%) were cured or improved The results of surgery in thi arbitra ily defined group of patients pre enting ome indication of abnormal renin secretion were ignificantly better than in the remaining 3 patients showing normal renin parameter (Fisher exact te t $p < o\ o5$)

Conclusion:

A succesful outcome of surgical therapy has been obtained in 86% of this series of hypertensive patient The peroperative and early postoperative mortality was about 10% In our view the e figures are encouraging Surgical therapy of hyperten iv di ea e is certainly worth-while but very careful election of patient is necessary

Postoperatively the fundus was normal in 6 patients and minimal hypertensive lesions were still present in 4 patients The ECG showed left ventricular strain and/or left ventricular hypertrophy in 5 out of 10 patients preoperatively Po toperatively the ECG was normal in all patients but one in whom signs of left ventricular hypertrophy persisted

Eleven of the patients had no symptoms One patient continued to have periods of headaches and in this case normotension was not achieved postoperatively These 12 patients all reported having observed increased (9) or unaltered (3) capacity for work The thirteenth patient was the patient with persistent subnormal production of aldo sterone Allthough the blood pressure was normal the patient continued to be very tired Hyperkalemia and moderate renal insufficiency was seen Despite substitution with fludrocortisone the subjective symptoms continued and 2 years after the operation the patient suffered a cerebral hemorrhage The blood pressure remained normal

Summary

Thirteen patients were followed for 4 46 months after removal of an aldo terone producing adenoma

Normotension wa achieved in all cases but two in whom moderate diastolic hypert nsion was easily managed on diuretic therapy

All were cur d of hypokalemia and symptoms related to low plasma potassium

Per i tant selective hypoaldosteronism was seen in one patient

A gr tifyi g regre sion of symptoms and signs related to art rial hypert n ion was seen

Medical treatment with aldosterone antagonists may cure the pati nt to the same extent as surgery The present results encourage the use of urgical tre tment in these young pati nts ince a life-long drug therapy - with its ttendant problems is the only alternative

combined finding of subnormal-low normal plasma renin concentration high normal-increased excretion of tetrahydroaldosterone and autonomous aldosterone production In 10 cases the localization of the suspected adenoma was carried out by demonstration of unilateral aldosterone efflux at venous catheterisation and/or demonstration of unilaterally increased uptake of ^{131}I-cholesterol at scintiphotography

Surgery

Unilateral adrenalectomy was performed in 12 cases and extirpation of a solitary adenoma in one case In all the patients a solitary adenoma was found The peroperative mortality was zero

Follow-up

Ten of the patients were examined by the authors Three of the patients were unable to participate However medical records were available for these patients and these data are included in the present report Blood and urine samples were obtained from all patients The observation time was 4-46 months (mean 19 months) All the patients were examined without any antihypertensive or diuretic treatment

Results of the follow-up study

The mean blood pressures were 93-132 mm Hg (mean 109 mm Hg) In all cases a decrease from the preoperative values was found In two cases the mean blood pressure decreased 15 and 19 mm Hg respectively and in the remaining cases the decrease in mean blood pressure was 20-53 mm Hg In two subjects only the diastolic BP remained above 100 mm Hg Plasma renin concentration was 18-61 μGU/ml and excretion of tetrahydroaldosterone 4-53 μg/24 hours Serum potassium was 3 8-5 3 mEq/l In one patient hypoaldosteronism and hyperkalemia developed and persisted after operation In 10 patients the fundus of the eye was evaluated preoperatively as well as postoperatively Preoperatively only two patients had normal fundi and 8 patients various degrees of hypertensive retinal lesions (grade II-III according to the Keith-Wagener classification as a maximum)

The special objectives of the hypertension programme are

1 TO FIND FOR TREATMENT GREATEST POSSIBLE NUMBER OF HYPERTENSIVES

2 TO KEEP IN CONTROL GREATEST POSSIBLE NUMBER OF THE PATIENTS

3 TO UNIFY THE DIAGNOSTIC AND THERAPEUTIC METHODS OF THE PHYSICIANS

4 TO GATHER NEW INFORMATION ABOUT OCCURENCE OF HYPERTENSION AND FUNCTION OF HEALTH SERVICES

The programme include screening organizing of services health education to the public training of personnel and information services A hypertension register is the major tool of ensuring the follow-up of the patients In the programme area everybody with elevated blood pressure val es is registe ed The regi tration like the follow-up and treatment is done though the normal h alth services of the county The register also s rve the evaluation of the programme together with the baseline the follow- p and the terminal surveys (4)

In the baseline situation in 1972 22 per cent of the 25-59 year old popul tion had h h blood pre sure The age-specific prevalence rates are shown in the FIGURE 1

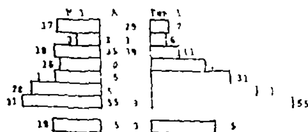


FIGURE 1 Preval s of hypertensive 1) according to age and sex among th middle aged popul tion in orth Karel a
1) Blood pressur 160/95 mmHg aged 30-59
≥150/90 mmHg aged 25-29

The tr tme t of hype te sio w s nsatisfactory shown in many other count i Half of the hype t nsives (54%) were not aw re of thei cond tio h lf of those awa e (47%) we e neve treated half of the tr ted (5) ha d o t nued the treatment and les than half of the tr t d (30%) subjects w re normotensive

Some p n n lt

During th fi t 3 5 ye s of th programme (197 -7) th blood pre sure

PRINCIPLES AND EXPERIENCES WITH A PROGRAMME FOR COMMUNITY CONTROL OF HYPERTENSION AS PART OF THE NORTH-KARELIA PROJECT

Jaako Tuomilehto, Aulikki Nissinen and Pekka Puska

North Karelia Project, Coordinating Cetre, University of Kuopio, Box 40, 70101 Kuopio 10 Finland

The hypertension programme of the North Karelia Project is a substantial component of a comprehensive programme for community control of cardiovascular diseases in the county of North Karelia - a community of 180 000 inhabitants in Eastern Finland (?) At the same time it is a subprogramme of WHO programme for community control of hypertension (5) The background and the basic principles of the whole North Karelia Project (1) as well as those of the hypertension programme (3) are described elsewhere

The main objective of the hypertension programme is reduction of high blood pressures among the whole population of North Karelia Table 1 gives the basic characteristics of the programme

MAIN OBJECTIVE	COMMUNITY CONTROL OF HYPERTENSION TO REDUCE ITS COMPLIC[illegible]TIONS
ENDPOINTS FOR EVALUATION	IMPROVEMENT OF TREATMEN[illegible] [illegible] THE COMMUN[illegible] REDUCT[illegible] OF CVD MORTAL[illegible]TY AND MOR[illegible]DITY IN [illegible] COMMUNITY
DURATION OF THE STUDY	5 YEARS (1972 1977)
INTERVENTION AREA	[illegible] POPULATION [illegible] 000 ALL AGES
REFERENCE AREA	GENERAL POPULA[illegible]ON [illegible] 000 A[illegible] AGES
DEFINITION OF HYPERTENSION	≥150 [illegible]/OR >90 (29 yea[illegible] ≥1[illegible] A[illegible]D/OR ≥95 (30 64 years) ≥1[illegible] [illegible]/OR ≥95 (6[illegible] ye[illegible]) [illegible] MEASUREMENTS
CASE FINDING MADE BY	HEALTH CENTRE PHYSIC[illegible] OCCU[illegible]NAL PHYS[illegible]IANS PUBLI[illegible] HE[illegible] NURSE [illegible]
INTERVENTION AND REFERENCE POPULATIONS	[illegible] FOLLOW-UP [illegible] PROTOCOL OF CLI[illegible]ICAL TREATMENT
TREATMENT PRESCRIBED BY	HEALTH CENTRE PH[illegible] [illegible] OCCU[illegible]TIONAL PHY[illegible]IANS
EDUCATIONAL MEAS[illegible]RES [illegible] TOWARDS	[illegible]

Table 1 Community control of hypertension in North Karelia

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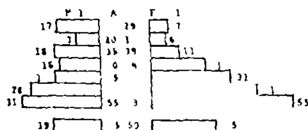


FIGURE 1 Prevalc e to of hype ten ives 1) according to age and se among the middle-aged populatio in North Karelia
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Some p n ... lt

During the first 3 5 ye of the programme (197 75) the blood press re

has been measured for nearly 90 of the middle-aged population The percentage of persons whose blood pressure was measured during the last 2 years in 1972 was 73 % among women and 53 % among men; the correspond figures in 1975 were 87 % for both sexes respectively

The percentage of middle-aged men under antihypertensive treatment increased from 3 to 10 the increase for women being from 9 to 14

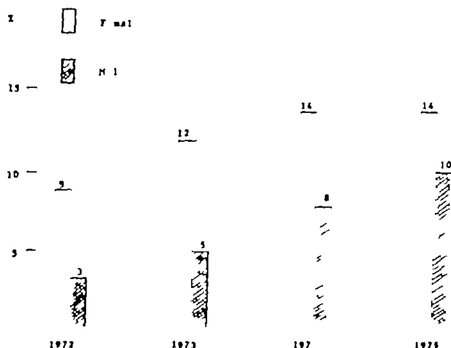


FIGURE 2 Development of antihypertensive drug treatment among the middle-aged population in North Karelia in 1972-75

In spring 1976 after 4 years intervention the number of patients in the hypertension register was 16 100 which was 9 1 per cent of the total population of North Karelia

At the end of the year 1972 there were 2 639 patients registered At the one-year follow-up the patient adrehence was good: the drop-out rate from the register was 9 per cent and the reasons for drop-out were mostly death failures of the completing and mailing of the record forms and not failure in the co-operation of the patients Only 1 per cent of the registered patients refused to co-operate

Among the registered patients the percentage of subjects with systolic blo pressure under 160 mmHg increased during the one-year follow-up period fro 11 to 27 The increase was greated among men than women The mean change of systolic blood pressure was -15 mmHg A decrease of more than 10 mmHg was observed among 53 per cent of all registrants majority of them

under drug treatmont already at the tino of the iritial reoistration

The percentage of subjects with diastolic blood pressuros under 95 mmHg increased from 14 to 34 The increase was again greated among men than women The moan change of diastolic pressure wa -6 mmHg A decroaso of more than 10 mmHg was observed among 43 per cent of tho registrants The percentage of normotensive subjects in the register increased from 9 to 20

Conclusions:

The presented experienc s from the hypertension programme of the North Karelia Project are encouraging

1 It has been possible to carry out the measurements of blood pressures in the whole population in the existing health services

2 Almost all hypertonsives - 9 per cent of tho total population - have been regi tered in the hypertension register during 4 years intervention

3 Antihyperten ive drug treatmont has improved especially among the middle-aged men

4 Tho number of drop-outs in the follow-up of the register has been minimal 99 per cent of the patients have boon co-operative

5 The blood prossure level of the patients has roduced and the proportion of normotensive subjects has increased significantly among treated patients

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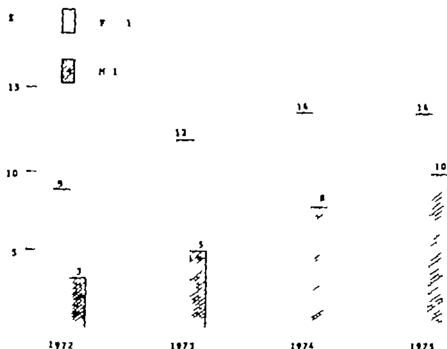


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Prospecti e study

The population sample of women as re-studied six years later during the years 1974 75 when the women were in ge strata between 44 and 66 years Altogether 1302 women were studied on this occasion which means 89 % of those studied in 1968-69 Nine women had during the interval between the two studies suffered myoc rdial infarction si of whom were survivors and 42 women who did not report a history of angina pectoris i the first study reported such symptoms on the second occasion Art rial hype tension as i the prospective study defined as antihypertensive treatment at the time of the first study i respective of the blood pres ure level or systoli blood pre sure withi the upper 5 per cent of each age st atum irrespective of whether being on antihypertensive tre tment or not In this was 126 women we e defined as hypertensives

RESULTS

According to the esults from the retrospective study (Table I) about half of the women who suff red myocardial nf ction had a history of arteri l hypertenion compared to about one fourth of the women who reported a history of angina pectoris and 12 % of women in a ref rence group i the population sample of the same ages

TABLE I History of arte ial hypertension i women with ischemic heart disease compared to women in the general population of the same ages

	Retrospe tive study		Prospe ti e study	
Myocard l f rction	22/47	47 %	4/9	56 %
Angina pecto i	8/29	28 %	10/42	24 %
Referenc group	71/578	12 %	71/578	12 %

The figures are i agreement with the result obt ined from the prospective tudy (Tabl I) four out of ne women who had a myocardial i fa ct were cla si fied as hype tens ves at the fi st study Thi corresponds to an annual incidence f myocard al nfarct on f 5 3 pe 1 000 women i the *hypertensive women compared* to 1 0 i the popul t on sampl as a whole wh ch means five-fold increase

I sp te f an ncreased ncidence of myocardi l i farction in hypertensives the ove ll mo tality in this group was low Two of 126 women who were classified as hype tensives t the first study died during the six-ye interv l both *from* myocardi l farct on This means an annual mo tality of 2 6 pe 1 000 women in the hype tens es ompared to 3 0 in the population sample as a whole

2

ARTERIAL HYPERTENSION AS A RISK FACTOR FOR ISCHEMIC HEART DISEASE IN WOMEN – RESULTS FROM A RETROSPECTIVE AND FROM A PROSEPCTIVE STUDY

Calle Bengtsson Department of Medicine II University of Göteborg Göteborg Sweden

ABSTRACT Results from retrospective and prospective studies of women in Göteborg Sweden indicate that arterial hypertension is a risk factor for ischemic heart disease in women and of similar importance as in men

Myocardial infarction is more common in men than in women There exist a number of so-called risk factors for ischemic heart disease It might be supposed that the higher incidence of myocardial infarction in men is due to a larger number of risk factors for myocardial infarction in men It seems however that this is not the case There are no differences of importance between the two sexes concerning factors such as blood pressure serum cholesterol serum triglycerides or diabetes (1) There is a sex difference in smoking habits but this difference cannot be the only explanation It also seems that the same risk factors operate and are of similar importance in men as in women (1 3)

Risk factors for ischemic heart disease have been studied in the male (10) as well as in the female (1) populations of Göteborg Sweden In the present paper the rôle of arterial hypertension for the development of ischemic heart disease in women is discussed This discussion is mainly based upon retrospective and prospective studies of women in Göteborg

MATERIAL AND METHODS

Retrospective study

In 1968-69 a population sample of altogether 1462 women was studied in Göteborg Sweden (2) Women who gave a history of angina pectoris (n=29) according to a questionnaire (9) were compared with the other women in the population sample of similar age (women in the age strata 50 and 54 years n=578) as to history of arterial hypertension Only two women in the population sample had suffered myocardial infarction All women of the same ages who had an acute attack of myocardial infarction in Göteborg during the years 1968 70 were therefore included in the study (1) This was possible as all subjects with myocardial infarction in the area were registered in a myocardial infarction register (4) Arterial hypertension was in the retrospective study defined as a blood pressure which had lead to antihypertensive treatment prior to the population study or the attack of myocardial infarction

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DISCUSSION

The results from the present studies indicate that hypertension is a risk factor for ischemic heart disease in women The incidence of myocardial infarction is lower in hypertensive as well as non-hypertensive women compared to hypertensive and non-hypertensive men The results from the present studies agree with those from others e g the Framingham Study in which arterial hypertension coincided with an increased incidence of myocardial infarction and angina pectoris (7) and seemed to be as important a risk factor in women as in men

In spite of an increased incidence of myocardial infarction the overall prognosis of hypertensive women seemed to be good as judged from the present studies These and similar observations from other studies might bring the question to attention whether antihypertensive treatment should be recommended in hypertensive women The low incidence of myocardial infarction in women has also made controlled studies regarding the effect of antihypertensive treatment less rewarding in women The most important information on the effect of antihypertensive treatment obtained up to now refers to the Veterans Administration Studies (11 12) but concerns only men A significant decrease in stroke was noted while the effect on ischemic heart disease was doubtful

The present population sample was too small to allow conclusions concerning the rôle of hypertension for the development of stroke in women In the Framingham Study however similar incidence of stroke was found in women as in men and in hypertensive women as in hypertensive men (6 8) Signs of left ventricular strain were also found as common in hypertensive women as in hypertensive men (5)

Summarizing our knowledge today we thus know that antihypertensive treatment is effective in the protection from stroke in men and there is no reason to believe that it should not be the case also in women The protection from myocardial infarction is more doubtful Vascular disease of the brain is as common in women as in men It therefore seems logical to treat women according to the same principles as men The overall good prognosis of the hypertensive women in the present series might also speak in favour of antihypertensive treatment as almost all of the antihypertensive women had received effective antihypertensive treatment

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a/H-r tio f om the apexcardiogram and atrial sound from the phonocardiogram

An a/H-ratio above 15 per cent was considered abnormal and the proportion of hyperte sives with bnormal a-wave was 39 per cent which was signifi antly higher than the corresponding proportion i the refer nc group 6 per cent

An abnormal trial sound is said to have the same haemodynamic ig ificanc s an abnormal a-wave in the apexc rdiogram Good corr latio has been how betve n the relative amplitude of the a-wave and index of the l ft ve tricular distensibility There f re an abnormal a-wave i the ape cardiogram and an abnormal atrial sound were both r g rded s igns of decr ased l ft ventricular distensibility The proportion of hypertensives with abnormal t i l sounds was 26 per c t which was signific ntly higher than the o responding p oportion i the reference group 3 per ce t

Casual nd re ting blood p s u i r lati n to sig s of l ft ve t ic l hype t ophy on rthogonal ECG and sig s of d r ased l ft ve tricul r di tensibility

No hypert nsi xhibited increased QRS-amplitud s on orthogonal ECG nd t the ame time a abnormal a-wave or abnormal atrial sound Thi wa i co tr st to what we had expected and to finding in previous studi s i thi fi ld One reason for this ight be that the hypertrophy was not of such a degre as to give a suffi i t incre se i left ve tricular wall thickness to give disten ibility hange The deg ee f hypert ophy i ou hypertensives was p obably mild ompared to the hypertrophy i aortic stenosi or the othe form of pronounced hypertrophy i previous tudies

The signs of decreased left ventricule di te sibility in the hypert si group could not be lated to hypertrophy at lea t not l ctrocardiog aphically p ov n no ould they be related to ischaemi heart di s (IHD) i ce no subject had igns o symptom f IHD A structurally normal ventricle may hibit the same low diste ibility as scarred o hypertrophied ve tricle if its filling p re i levated suffi i tly Inc ea ed centr l blood volume has bee desc ibed i ea ly esse ti l hypertension One planation for the sig s of decre sed l ft ventric la di t nsi bility might be abnormal dist sion of the left ve tricle during t i l ont action partly due to increased ce t al blood volume

NON-INVASIVE ASSESSMENT OF CARDIAC FUNCTION IN NORMOTENSIVE AND HYPERTENSIVE 50-YEAR-OLD MEN

By

John Wikstrand Göran Berglund Lars Wilhelmsen and Ingemar Wallentin

From the Department of Clinical Physiology and the Section of Preventive Cardiology Department of Medicine I Sahlgren s Hospital University of Göteborg Sweden

The prevalence of signs of heart involvement was studied non-invasively with orthogonal and conventional ECG apexcardiogram carotid pulse tracing and phonocardiogram in a group of untreated hypertensives (n=35) and a reference group (n 73) All were derived by screening a random population sample of 50-year-old men The diagnosis of essential hypertension was based on casual blood pressures above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions ECG was performed in all subjects The other variables were studied in a randomly selected half of both the reference and the hypertension group

RESULTS AND DISCUSSION

Blood pressure and heart rate

The mean values for casual blood pressure were 197/119 mm Hg in the hypertension group and 142/92 mm Hg in the reference group and the casual heart rate were 84 and 75 beats/min The mean values for resting blood pressure were 154/96 mm Hg in the hypertension group and 123/77 mm Hg in the reference group and the mean values for resting heart rate were 61 and 60 beats/min

Electrocardiography (ECG)

The scalar ECG leads X Y and Z were simultaneously recorded in accordance with the Frank s corrected orthogonal lead system The following criteria for left ventricular hypertrophy were used: R_x over 1 8 R_z over 1 3 or $R_x + S_y$ over 1 9 mV The amplitude limits correspond to the upper 97 5th percentile in the reference group Conventional ECG was coded in accordance with the Min esota code and a combination of amplitude criteria a d S-T or T criteria were used The proportion of hypertensives with signs of left ven tricular hypertrophy on orthogo al ECG was 33 per cent which was significantly higher than on the co ventional ECG 9 per cent

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The sign f decreased left ve tricul r dist nsibility i the hypert sion g oup could not be related to hypertrophy at l st not lectrocardiographi ally prove nor could they be related to ischaemi heart dise s (IHD) since no subj ct had igns or symptoms f IHD A structu lly normal ntricl may xhibit the same low di ten ibility s sco ed o hypertrophied ve tri l if its filling p s r i l ted ffici tly Increased central blood volume ha be d scribed i e rly e e ti l hypertension One expl nation for the sign of d creased left ve t iculer diste sibility ight be bnormal di te sion f the left ventricle during tri l ontraction partly due to increased central blood volume

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Tabl 1
The proportio of subj cts in hypertension and reference groups with igns of left ventricular hypertrophy (LVH) o orthogonal and conventional el ctrocardiogram (ECG) abnormal a-wave i the apexcardiogram bnormal atrial sound in the phonoc rdiogram and prolonged interval between the aortic component of the second heart sound (A_2) and the opening point (O) in the apexcardiogram respectively

	Hyperte sio group %	Reference group %
Orthogonal ECG (LVH)		
R > 1 8 $R_x + S_y > 1\ 9$ or $R_z > 1\ 3$ mV	33	3
Conve tional ECG (LVH Minnesota Code)		
3:1 or 3:3 and 4:1-3 o 5:1-3	9	3
Apexcardiogram		
a/H > 15%	39	6
Phonocardiogram		
Abnormal atrial sound	26	3
Prolonged relaxation time (or prolonged early filling phase?)		
$A_2O \geqslant 150$ ms	56	3

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Blood pressures were measured after one hour s rest and compared to the casual values Resting blood pressure became normal after one hour s rest in the hypertensives with signs of decreased left ventricular distensibility but not in the hypertensives with signs of left ventricular hypertrophy This might suggest that the former group is in an earlier phase of hypertensive disease while the latter group is in a later phase Whether patients in the former group will pass over into a hypertension of the latter type or not must be studied prospectively In advanced left ventricular hypertrophy an abnormal a-wave in the apexcardiogram is a very common finding It might be that an initial abnormal a-wave caused by stiffness due to increased volume of the left ventricle can eventually become normal and then, when the left ventricular hypertrophy becomes pronounced or fibrosis is added structural changes in the left ventricular wall can again give rise to an abnormal a-wave

Total electromechanical systole (QA_2) left ventricular ejection time (LVET) and the isovolumetric contraction time (ICT)

There was no significant difference in total mechanical systole QA_2 left ventricular ejection time LVET or the isovolumetric contraction time ICT between the reference and the hypertension group Since the blood pressures are higher in the hypertension group the figures imply increased contractility and an increased rate of ejection in the hypertension group

The interval between the aortic component of the second heart sound (A_2) and the O-point in the apexcardiogram

Only one subject in the reference group had an A_2O interval over 150 ms In the hypertension group 56 per cent had an A_2O interval above 150 ms It is said that this interval depends on heart rate blood pressure and age This could not explain the differences in these two groups It has been shown that the A_2O interval includes in addition to the relaxation time a short period of the early filling phase of the left ventricle We have however so far no explanation for the prolonged A_2O interval in the hypertensives We have found in the majority of patients in a non-invasively studied infarction group the same finding of a prolonged A_2O interval It is possible that ischaemic heart disease plays a roll for this interval as maybe also for the a/H-ratio

Tabl 1
The proportion of subjects in hyperte ion and ref re ce groups with igns of left ventricul r hypertrophy (LVH) on orthogonal and conventional lectrocardiogram (ECG) abnormal a-wave in the apexcardiogram abnormal atrial sound in the phonocardiogram and prolonged i terval between the aortic component of the second heart sound (A_2) and the opening poi t (O) in the apexcardiogram respectively

	Hyperte sion group %	Reference group %
Orthogonal ECG (LVH)		
$R_x > 1$ 8 $R_x + S_y > 1$ 9 or $R_z > 1$ 3 mV	33	3
Conventional ECG (LVH Minnesota Code)		
3:1 r 3:3 and 4:1-3 or 5 1-3	9	3
Apexcardiogram		
a/H >15%	39	6
Phonocardiogram		
Abnormal atri l sound	26	3
Prolonged relaxation time (or prolonged early filling phase?)		
$A_2O \geq 150$ s	56	3

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sympathetic effects of electrical stimulation of the aortic nerve distal to the blocking site

A steep 20 mm Hg rise in mean arterial pressure to a plateau maintained for 5-10 sec caused severe sympathetic inhibition when mediated via the intact left aortic nerve and no changes in sympathetic discharge when the A fibres were blocked Effects via C fibres occurred with higher pre sures however and a rise of 50 mm Hg in mean arterial pressure from resting values of 70-109 mm Hg produced a 60 % reduction in sympathetic ctivity (average from 10 rabbits) This degree of sympathetic inhibition was about the same as that obtained by electrical stimulation of aortic nerve C fibres with 4 imp/sec

Receptors with C fibres in the aortic or carotid sinus nerves are known to be excited by local application of catechol mines to baroreceptor areas (4 5 8) probably also by i v administration (2) Confirming our previous observa tions (2) aortic nerve C fibres during i v infusion of noradrenaline (5 µg/kg min) were found to transmit information about rises in pressure which prio to the infu ion had no effect on the receptors with C fibres

Our studies show that on the afferent side the baroreceptor eflex is effected by two different receptor sy tems receptors with myel nated fibre which ar oper tive at normal pressu s and receptors with non-myelinated fibres For the first time barorecepto C fibres have been shown to in duce reflex re ponses only to pressures above normal levels; they thus play a major role in cardiac nd vasomotor control at ac te hypertensive pressures Judged by the response to nor drenaline the rec ptors with C fibres might also serve the substrate for efferent sympathetic regulation of the baro eceptors

Much still remains unknown about the baroreceptor C fibres - g the r functio in chro ic hype tension This study is an introduct on to the role of th se receptors in cardiovasul r co trol

The Role of Non-myelinated Afferents in the Baroreceptor Reflex

Harald Aars Lisbeth Myhre and Bruce A Haswell
From Department of Physiology and Biochemistry Dental Faculty University of Oslo Oslo Norway

The baroreceptor reflex – the excitation of vagal cardiac neurons and inhibition of sympathetic activity in response to a rise in arterial blood pressure – is an extremely important regulator of cardiovascular functions Our knowledge of the afferent limb of this reflex the baroreceptors and their fibres is limited to myelinated baroreceptor fibres (A fibres) Of the equally numerous non-myelinated afferents (C fibres) in baroreceptor nerves we only know that when electrically evoked activity in these fibres causes largely the same reflex effects as stimulation of A fibres at higher frequencies (3 6 7) Little is known however of the normal function of the C fibres e g of whether and to what extent they contribute to the baroreceptor reflex Our previous work indicated that baroreceptor C fibres do not participate in the reflex bradycardia resulting from a moderate pressure increase (1) To study the problem more fully and with a more sensitive measure of reflex activity we have compared in anesthetized rabbits the sympathetic responses to a pressure rise when mediated by intact afferent baroreceptor pathways and by C fibres only

Sympathetic activity was recorded in the left renal nerve and blood pressure was increased by inflation of an aortic balloon The baroreceptor pathways were restricted to the left aortic nerve by occlusion of both carotid arteries and sectioning of the right aortic nerve Separation of effects via A and C fibres in the left aortic nerve was achieved by intermittently blocking the conduction through the A fibres with anodal block To this end a small current usually about 5 µA was passed through the nerve between two electrodes 2–3 mm apart Higher currents blocked both types of fibres The precision of the block was controlled by studying the evoked action potentials in the aortic nerve and the

sympath tic effects of electrical stimulation of the aortic nerve distal to the blocking site

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Plasma renin plasma aldosterone exchangeable sodium and renal hemodynamics in normotensive and hypertensive recipients of a kidney transplant with and without transplant renal artery stenosis

H J Kornerup and E B Pedersen Medical Department C Kommunehospitalet Arhus Denmark

Blood pressure (BP) plasma renin concentration (PRC) plasma aldosterone concentration (PAC) exchangeable sodium (ES) renal plasma flow (RPF) and glomerular filtration rate (GFR) were studied in 7 normotensive patients (group 1) 7 hypertensive patients without transplant renal artery stenosis (TRAS) (group 2) and 5 hypertensive patients with angiographically verified TRAS (group 3) after successful kidney transplantation Hypertension in group 2 was characterized by a normal PRC and PAC on a liberal sodium intake a normal response of PRC and PAC on a fixed low (10 mEq/day) and high (150 mEq/day) sodium intake a significantly lower ES compared with the normotensive group on a fixed low sodium intake but not on a fixed high sodium intake and a positive correlation between mean BP and ES on both sodium regime In contrast hyperten ion in group 3 was characterized by a normal to varyingly increased PRC on a liberal sodium intake a reduced response of PRC on sodium restriction a positive correlation between PRC and PAC on a liberal sodium intake and an ES which was not significantly different from ES in the other patient groups In one patient i group 3 who underwent urgical correction for TRAS PRC and PAC decr ased before operation during sodium restriction but BP remained high ntil aft r operation when BP normalized simultaneously with a decrease i ES RPF nd GFR were significantly lower in group 2 compa ed with the normotensive group whereas a lowe RPF and GFR in group

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Plasma Catecholamines and Arterial Hypertension

N J Christensen

Medical Department I Amtssygehuset & 2nd Clinic of Internal Medicine Kommunehospitalet Arhu Denmark

Plasma noradrenaline s an index of sympathetic nervous activity Th relationship between plasma noradrenaline (NA) and blood pressure w s studied in tetraplegic patients with physiologically complete cervical pinal cord transections abov the level of the sympathetic outflow (10) These patients have no supraspinal control of their sympathetic outflow Neurogenic hypertension may be ndu ed by visceral and muscle stimulation This hypertension is a result of reflex sympath tic ctivity through the isolated pinal cord Plasma NA nd pl sma adrenaline (A) were measured by a sensitive and precise double-isotope de vative a say (2)

In the tet aplegic th verage resting blood pressure was 107/59 mm Hg hea t ate w s 66 beats/min and plasma NA and A levels were 0 05 and 0 005 ng/ml respectively In the controls average blood pre sure was 117/79 mm Hg heart rate wa 61 be t /min nd r ting plasma NA and A level were 0 20 nd 0 06 ng/ml r spectively Re ting blood pressure nd plasma NA nd A levels were ig ificantly lower in the tetraplegics ompa ed to the controls

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the isolated spinal cord Plasma NA rose significantly from 0 05 to 0 16 ng/ml There was a significantly linear relationship between plasma NA and mean blood pressure

In the tetraplegics infusion of l-NA to raise the blood pressure to comparable levels (from 105/58 to 183/93 mm Hg) resulted in plasma NA levels 10-30 times higher than during muscle and bladder stimulation

The evidence indicates that tetraplegic patients have lower resting arterial blood pressure and plasma NA and A levels when compared to normal subjects and this reflects diminished resting sympathetic nervous activity In these patients the rise in blood pressure following increased sympathetic nervous activity was consistently accompanied by an elevation in plasma NA The hypertension was not secondary to the rise in plasma NA Plasma NA in these patients may be regarded as a useful index of prevailing sympathetic nervous activity

Plasma renin activity and plasma NA in tetraplegic patients: Plasma renin activity and plasma NA were studied in four tetraplegic patients before during and after head-up tilt to 45° (9) Ten minutes after tilting plasma NA and A rose significantly in the controls but remained unchanged in the tetraplegics All tetraplegic patients had a fall in arterial blood pressure during tilting The resting plasma renin activity was above normal in the tetraplegics nad rose more quickly and greater on head-up tilt than in published studies of normal subjects It is likely that renal baroreceptors are important in the control of renin release in the tetraplegic patients

Plasma catecholamines in essential hypertension Most patients with labile and fixed hypertension have normal urinary excretion r tes of catecholamines Some authors have reported elevated plasma noradrenaline concentrations in patients with essential hypertension but in these studies the effect of age on plasma NA has not been taken into account (4 5 8) In a study of 21 patients with essential hypertension and 32 age-matched control subjects the relationship between pl sma noradrenaline and age was the same in patient with and without esential hypertension (11) It s possible that plasma catecholamines are altered in smaller groups of patients with essential hypertension especially in pat ents with low and high plasma renin and these possibilities deserve further investigation Some patients with labile hypertension are haracterized by elevated urinary excretion rates of adrenaline (Chr stensen In preparation) Plasma c techolamine are normal in patients with renal hypertension (1)

Propranolol and sympatheti nervous ctivity Propranolol increases sympathetic nervous activity and plasma NA and A levels (3 6 7) The mechanism by wh ch propr nolol increa s plasma NA does probably not involve th baroreceptо It is mo t likely due to the decrease in cardia output and an altered m tabolic stat in the tissu (oxygen or related factors) and induced by afferent neural signals from the ti sues (Hes e Hans n & Chris tens n Submitted for publication) The rise in plasma A after propranolol is mo t pronounced du ing prolonged exercise and at least partially due to an exagger ted decrea e in blood glucose d ing exercise (7)

Clonidine and sympathetic nervous activity: Clonidine reduces ympathetic nervou activity In tetraplegic patients neuro-g nic hypertension induced by bladder stimulation and cau ed

by reflex sympathetic activity through the isolated spinal cord is completely inhibited by i v injection of clonidine and at the same time the rise in plasma NA is abolished The effect of clonidine is therefore not dependent on supra-spinal control of the sympathetic outflow (Mathias & Christensen: In preparation)

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HAEMODYNAMIC STUDIES IN YOUNG MEN WITH MILD BLOOD PRESSURE ELEVATION

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Abstract The cardiac output at rest and the intra-arterial blood pressure and hand blood flow at maximal vasodilation were determined in two groups of men aged 18-22 years 44 patients were referred from a military enlistment centre because of mild blood pressure elevation and 29 normotensive volunteers were mainly recruited from the same enlistment centre In addition in a majority of subject in both groups the auscultatory blood pressure of both parents was determined

The patients were characterized by a significantly higher cardiac index at rest and a significantly higher vascular resistance in the hand blood vessels at maximal vasodilation than the controls indicating the pre ence of tructural alterations in the resistance vessels of these subjects with only very mild blood pressure elevation

The tendency to increased vascular resistance in the hand blood vessel at maximal vasodilation wa more prominent in patients with a normal cardiac index than in those with a high index This suggests inclusion in the patient group of tense anxious individuals with an elevated cardia index but otherwise normal circulation but does not exclude the po sibility that these pati nts may develop tructural v scular hange later on

The parents blood pre sure was higher in the group of patients both for patients with a normal and those with a high cardiac index compared to the parents of the controls

A hyperkinetic cir ulation t rest with an elevated cardiac output has repeatedly been found to be a group characteri tic in patients with latent borderline terial hypertension (1 5 7 9 10 13) Compared to findings in normot nsive individuals the calculated systemic vascular re istance in such patient i relatively elevated (6 8 11)

The high cardiac output at re t may be interpreted in two way ; ithe it i a h racte istic of patients with early essential hypertension or it is due to inclu ion of tens anxiou individuals with

a hyperkinetic but otherwise normal circulation

To further evaluate this the systemic haemodynamics at supine rest and the peripheral haemodynamics during physiologically induced maximal vasodilation were studied in young men with mild blood pressure elevation comparing the results with those from carefully matched controls

MATERIAL AND METHODS

Subjects Forty-four young men aged 19-22 years were referred from a military enlistment centre after having shown auscultatory blood pressures ≥150 mmHg systolic or ≥90 mmHg diastolic at a highly standardized routine medical examination Further inclusion criteria were systolic blood pressures at two subsequent follow-up visits of ≥140 mmHg No patient had any indication of organic changes in the cardiovascular system or in the kidneys

Twenty-four male volunteers aged 18-20 years were recruited from the same pool of young men at the military enlistment centre All of them had auscultatory blood pressures ≤130/≤80 mmHg at two separate examinations In addition five male blood donors aged 19-22 years fulfilling the same blood pressure criteria were included as controls

The auscultatory blood pressure in the afternoon was determined in both parents of 30 patients and 22 controls

The study protocol was approved by the Ethical Committee of the University and informed consent was obtained from all subjects participating in the study

Procedure The subjects reported at the laboratory in the morning after having had a light breakfast Under local anaesthesia a polythene catheter was introduced percutaneously into the brachial artery, and another catheter was advanced to a subclavian vein via percutaneous puncture of an antebrachial vein

After 45 minutes rest in the recumbent position the intra-arterial blood pressure heart rate and cardiac output were measured repeatedly during one hour of continued rest using pressor transducer recordings (Elema-Schönander EMT 35) and a dye-dilution technique (Cardiogreen® Cardiognost® Atlas) The average figures from this series of recordings will be presented

The subjects were then transferred to another room for determinations of their peripheral haemodynamic during a state of physiologically induced maximal vasodilation (12) After a period of general heating under an electric arc and local heating of both hands in water plethysmographs at a temperature of 43^{o} C arterial occlusion was applied to both arms and the subjects were a ked to exercise their hands until exhaustion Immediately after release of the occlusion the hand blood flow and the electrically integrated mean intra arterial blood pressure were determined The average values from three determinations of the blood flow at maximal vasodilation in the catheter-free hand have been used for the statistical analysis The va cular resistanc in the hand blood vessels at maximal vasodilation ha been derived in arbitrary units as the quotient between the m an brachial rterial blood pressure in mmHg and the calculated hand blood flow in ml per 100 ml tissu and minute

Stud nt s t test has been used for statistical analysis of differences between the groups

RESULTS

In addition to a signific ntly higher intra-arterial blood pressure the group of patients with mild blood pressure elevation also had a signi ficantly higher cardiac index at rest in recumbency compared to the controls (Fig 1) The vascular resi tance in the hand blood vessels t maximal v sodilation wa significantly higher in the group of patients than in the controls

Subdivision of the patient into those with a cardiac index above the control mean + 1 S D i e a hyperkinetic group and those with a cardiac index below the co trol mean + 1 S D i e a normokinetic group evealed that the tendency to increased vascular resistance in the hand blood ves els at maximal vasodilation was in comparison to the controls stati tically significant in the normokinetic group but not in the hyperkinetic bjects (Fig 2)

Compared to the parent of the control the uscultatory systolic blood pressure of the parent of the pati nts wa ignificantly higher both in the hyperkinetic patients and in the normokinetic group (Fig 3)

DISCUSSION

The present results once again confirm hyperkinetic circulation at rest to be a characteristic group finding in subjects with asymptomatic blood pressure elevation Further the study reveals that an increased vascular resistance in the hand blood vessels during a state of induced maximal vasodilation can be demonstrated even in young men with only very mild blood pressure elevation when compared with carefully matched normotensive controls This indicates the presence of restrictive changes in the peripheral vascular bed which cannot be overridden by potent physiological stimuli similar to those seen in cases of established arterial hypertension (12) even in what may be assumed to be the earliest stages of hypertensive cardiovascular disease

Thus patients with latent borderline arterial hypertension exhibit not only a hyperkinetic circulation at rest but also an increased vascular resistance in the hand blood vessels at maximal vasodilation However this tendency towards an increased vascular resistance was more prominent among individuals with a normokinetic circulation than in hyperkinetic subjects which might indicate inclusion in the patient group of tense anxious subjects who present at the investigation with an elevated cardiac index, but who otherwise have a normal circulation If this is true it does not support a dominant role of a hyperkinetic circulation for the development of manifest hypertensive cardiovascular disease However the present results do not in any way exclude the possibility that the hyperkinetic patients may in time develop restrictive vascular changes typical of hypertensive cardiovascular disease especially as the parents of these subjects also had an elevated auscultatory blood pressure level compared to the control parents Only long-term follow-up studies will provide an answer to this question

ACKNOWLEDGMENT

This study is receiving financial support from the Swedish National Association Against Heart and Chest Diseases

FIGURE LEGENDS

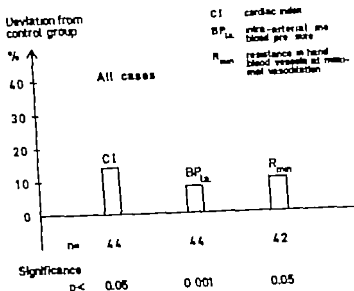


Fig 1 Differences in sy temic and peripheral haemodynamic finding in the total patient group of 44 young men with mild blood pressure elevation to the group of 29 normotensive controls

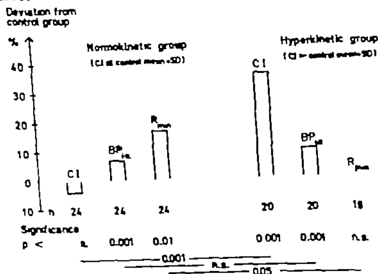


Fig. 2, Differences between patients and controls after subdi ision of the patients into a normokinetic and a hyperkinetic group For abbreviation ee Fig 1

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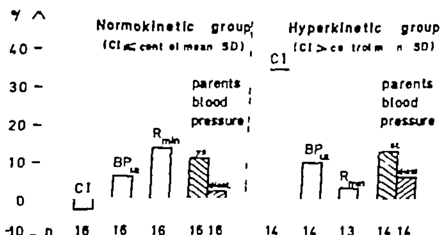


Fig 3 Parents auscultatory blood pressure in relation to the haemodynamics of patients and controls For abbreviations see Fig 1

the cardiac index should decrease and the resistance should increase over the years different from what is seen in normotensives So far no systematic long-term studies have been published This paper is a preliminary report on a hemodynamic restudy after 8-10 years in 28 young subjects with untreated essential hypertension

Material and methods

The first study included 33 normotensive controls and 77 subjects with essential hypertension of whom 36 were below 40 years and who will be discussed here n age group I (17-29 years old n) all in WHO stage I) the mean value of the intraarterial blood pressure in 1967 was 150/92 mmHg Two subjects were lost abroad Of the remaining l all were alive apparently healthy and still in WHO stage I; l6 have been untreated and 15 of these have been restudied

In ge group II (30- 9 years old n=17 all in WHO stage I) the mean value of the intraarterial pressure was 160/99 mmHg when first studied One subject died The remaining 16 patients we e alive ll still in WHO group I; 13 have been untreated and they have all been restudied

Exactly the same methods were used in both studies The subjects were tudied at rest sitting and during muscular exercise at 300 600 and 900 kpm/min Oxygen consumption was measured by Douglas bag and Scholander technique heart rate by ECG cardiac output by dye ilution method arterial blood pressure by catheter in the brachial artery (12)

HEMODYNAMIC TRENDS IN UNTREATED ESSENTIAL HYPERTENSION
PRELIMINARY REPORT ON A 10 YEAR FOLLOW-UP STUDY

LUND-JOHANSEN, PER, M D

Introduction

In a previous study from 1967 it was demonstrated that the hemodynamic mechanisms responsible for the increased blood pressure in essential hypertension differed in the various age groups (12) The total peripheral resistance at rest sitting fell within "normal" range in nearly all patients below 30 years, but was increased in almost all 40-49 years old During exercise, however, the total peripheral resistance was abnormally high in all age groups, including the youngest The resting cardiac index in the youngest age group was significantly higher than in normotensive age matched controls and higher than in hypertensives one or two decades older The high cardiac index was associated with an increased heart rate, the stroke index being normal The cardiac index was however, not out of proportion of the oxygen need since the oxygen consumption at rest was also increased and the $A-VO_2$ difference normal During exercise the cardiac index was no longer high, but normal or low in all age groups and the stroke index was subnormal In contrast, in the normotensive control subjects 20-49 years old there were no or only negligible differences between the hemodynamic patterns at rest and during exercise over the three decades

The results from cross-sectional studies in subjects with untreated essential hypertension seemed to indicate that

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The first study included 33 normotensive controls and 77 subjects with essential hypertension of whom 36 were below 40 years and who will be discussed here. In age group I (17-29 years old, n=19, all in WHO stage I) the mean value of the intraarterial blood pressure in 1967 was 150/92 mmHg. Two subjects were lost abroad. Of the remaining 17 all were alive, apparently healthy and still in WHO stage I; 16 have been untreated and 15 of those have been restudied.

In age group II (30-39 years old, n 17, all in WHO stage I) the mean value of the intraarterial pressure was 160/99 mmHg when first studied. One subject died. The remaining 16 patients were alive, all still in WHO group I; 13 have been untreated and they have all been restudied.

Exactly the same methods were used in both studies. The subjects were studied at rest sitting and during muscular exercise at 300, 600 and 900 kpm/min. Oxygen consumption was measured by Dougl bag and Scholander technique, heart rate by ECG, cardiac output by dye dilution method, arterial blood pressure by catheter in the brachial artery (12).

Results

A survey of the most important findings are shown in figures 1-,
The oxygen consumption (VO_2) at rest had decreased from 171 to 157 ml/min/m^2 ($p<0.05$) in age group I, but was almost unchanged in group II During exercise there were only small changes
The blood pressure showed remarkably small changes During rest there were no significant changes in systolic (SAP), diastolic (DAP) or mean (MAP) arterial pressures During 900 kpm/min exercise there was a significant increase in MAP from 133 to 141 mmHg in age group I and from 141 to 147 mmHg in group II
The cardiac index (CI) had decreased significantly in both age groups at rest as well as during exercise In group I the mean value decreased from 3 81 to 3 22 l/min/m^2 at rest, in group II from 3 64 to 2 81 l/min/m^2 During the 900 kpm/min load the mean value decreased from 11 10 to 9 4/ l/min/m^2 in group I and from 10 41 to 8 96 l/min/m^2 in group II In both age groups the reduction in cardiac index was associated with a significant decrease in stroke index (SI) at rest as well as during exercise
The heart rate (HR) at rest and during exercise showed only small and insignificant changes in group I but in group II there was a significant decrease in the resting value (from 80 4 to 71 3 beats/min) The total peripheral resistance index (TPRI) had increased significantly both at rest and during exercise in both age groups

Discussion and conclusion

The most important findings in the 1967 study (12) have been confirmed by others (1, 10 11, 16 17 reviews in 13, 14 1)

In the follow-up study it has been demonstrated that the expected changes in the central hemodynamics had indeed occurred In the youngest group the increased VO_2 had fallen close to the value in age group II 10 years ago The increased HR persisted but in age group II the resting HR had decreased In both age groups the reductions in SI and increases in TPRI were impressive and very consistent

In this preliminary report there is no place for a detailed discussion about the cardiovascular effects of aging versus aging plus increased blood pressure However several cross-sectional studies in healthy normotensive subjects have demonstrated that aging from the 20-ties to the 40-ties involves no or only negligible changes in the cardiac performance (2 4 8 9) Two long-term studies in hypertensives - one in treated subjects (3) and one of shorter duration (5) have shown similar trends as those in this study It is therefore reasonable to conclude that the 10 year duration of hypertension was responsible for the far greater alterations in the central hemodynamics than what should be expected from aging alone

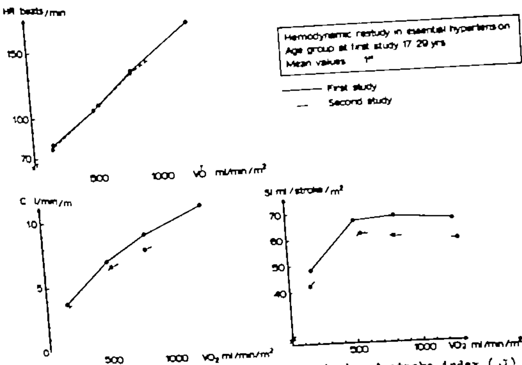
The changes show a remarkable similarity to what is seen in the spontaneously hypertensive rats when the time factor is expressed as a fraction of life duration (6 7 1 19) The findings seem to fit well with Folkow s theory about the restructuring of the high pressure compartments (7) The "vicious circle is operating in man but slowly and therefore hard to study

Results

A survey of the most important findings are shown in figures 1-3. The oxygen consumption (VO_2) at rest had decreased from 171 to 157 ml/min/m^2 ($p < 0.05$) in age group I, but was almost unchanged in group II. During exercise there were only small changes. The blood pressure showed remarkably small changes. During rest there were no significant changes in systolic (SAP), diastolic (DAP) or mean (MAP) arterial pressures. During 900 kpm/min exercise there was a significant increase in MAP from 133 to 141 mmHg in age group I, and from 141 to 147 mmHg in group II. The cardiac index (CI) had decreased significantly in both age groups at rest as well as during exercise. In group I the mean value decreased from 3.81 to 3.22 l/min/m^2 at rest, in group II from 3.64 to 2.81 l/min/m^2. During the 900 kpm/min load the mean value decreased from 11.10 to 9.4? l/min/m^2 in group I and from 10.41 to 8.96 l/min/m^2 in group II. In both age groups the reduction in cardiac index was associated with a significant decrease in stroke index (SI) at rest as well as during exercise. The heart rate (HR) at rest and during exercise showed only small and insignificant changes in group I, but in group II there was a significant decrease in the resting value (from 80.4 to 71.3 beats/min). The total peripheral resistance index (TPRI) had increased significantly both at rest and during exercise in both age groups.

Discussion and conclusion

The most important findings in the 1967 study (12) have been confirmed by others (1, 10, 11, 16, 17, reviews in 13, 14, 18)



Fi. 2 Cardiac index (CI) heart rate (HR) and stroke index (SI) at rest sitting and during ex r ise at tudy 1 and 2 Mean values Age group 1 - years at study 1

Legends to figures

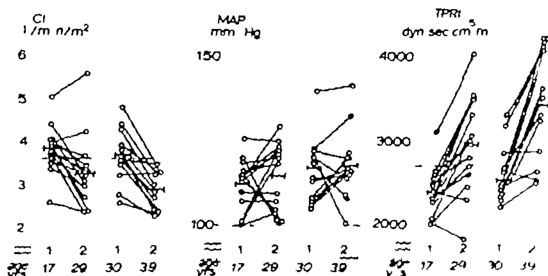


Fig 1 Cardiac index (CI), mean arterial pressure (MAP) and total peripheral resistance index (TPRI) at first (1) and second (2) study

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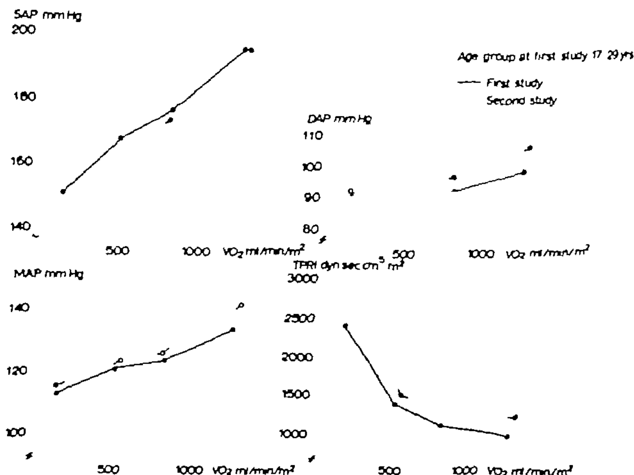


Fig 3 Systolic (SAP) diastolic (DAP) and mean arterial pressure (MAP) and total peripheral resistance index (TPRI) at rest and during exercise at study 1 and 2 Mean values Age group 17–29 years at stud 1

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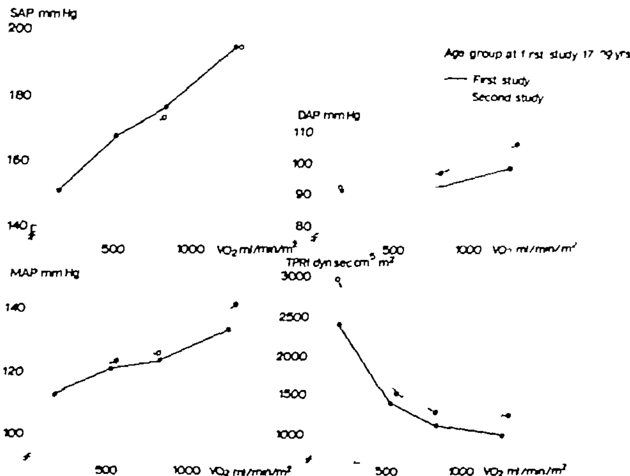


Fig 3 Systolic (SAP) diastolic (DAP) and mean arterial pressure (MAP) and total peripheral resistance index (TPRI) at rest and during exercise at study 1 and mean values Age group 17-29 years at study 1

PLASMA RENIN ACTIVITY SODIUM BALANCE AND SYMPATHETIC ACTIVITY DURING PROGRESS OF ESSENTIAL HYPERTENSION

By

Göran Berglund John Wikstrand and Lars Wilhelmsen
From the Departm ts of Medicine I and Clinical Physiology
Sahlgren s Hospital University of Göteborg Sweden

The mechanisms leading to the d velopment and maintenance of high B P in man re not known The ol of sodium balance plasm reni activity (PRA) and the sympatheti rvous system has been debated intensively in ecent years

The res lt presented her re based on recent studies i our group (1–3)

POPULATION

Both the material and the procedure of the inve tigations have bee described previou ly (1) Bri fly a 10% subsample of the 50–ye r-old mal population i Göt borg Sweden with B P below 160 syst lic and 95 mm Hg diastolic was selected as a normoten i group (n=41) All subject without antihypertensive treatment and ith systolic B P above 175 or diast li B P above 115 mm Hg on two separate occasions mad up the hyperte sion group (n=35)

METHODS

Plasma r i activity

No dierary salt i structions were given for the days preceding th investigation Blood sampl s for PRA determinations were drawn at 08 a m after 10 inut s upine re t PRA was determined according to Giese t al (6)

Sympatheti acti ity

Urinary noradr naline cretion was d termined separ t ly for day and night sing a modified method according to vo E l r and Floding (4)

Severity of hyperten i

I a r ndom half of two g oups re ting di tolic B P was det rmined phonographically and the height of the R-wave i l ad X on orthogonal ECG was cal ulated

The height f the R-wave i l ad X (R) of the colar ECG was shown to be the best singl di c iminator of left ve tricul r hypertrophy (9)

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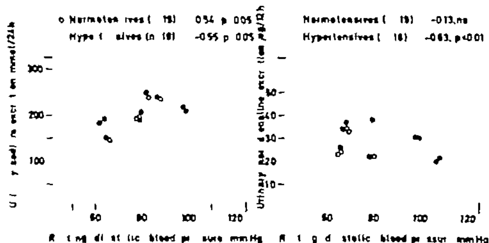
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Figure 2



The relationship between resting diastolic B P and urinary sodium xcretion nd urinary noradrenalin e reti n

Relatio ship of blood pressure to sodium cretio and sympathe tic activity

In th normoten ive group the re ting diastolic B P was positively co rel ted to inary sodium cretion (Fig 2) I the hypertensive group on the other hand there was a negative correlation The r ting diastolic B P was not significantly correlated with urinary noradr naline e retio during the day among the normote si s (Fig 2) but among the hype te ive a negative c relation was found Five hypert i e subj ct had a resting diastolic B P below 90 mm Hg These subjects had urinary sodium xcretion levels simil to the p ttern in the normote sive group and they had higher rinary norad naline xcretion d ring the day than the hypert sives who did not normalize their B P during rests This might indicate that the B P in th se five subjects wa maintained by a high ympoth tic tone and that they had not yet developed the p ttern with a low inary sodium excretion

DISCUSSION

Fig 3 pr sents hypothesis on the ol of the kidney i th development of essenti l hypert sio The hypothe is is an attempt to i t rpr t our result It is known from previou studi s that repeated incre ses of ympatheti discharge due to for example psychologi al stress l ad to pe t inc ases of B P

RESULTS

Plasma renin activity

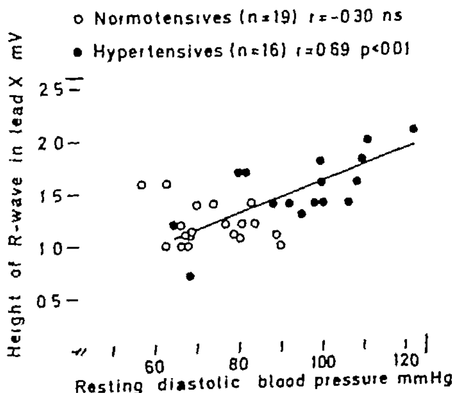
Both in the normotension and in the hypertension group PRA was roughly normally distributed with a slight scewness to the right Mean values were 0 78 and 0 65 respectively the difference being almost statistically significant (0 10> p>0 05)

Hypertensive subjects with low PRA (standardized for sodium excretion) had a significantly lower glomerular filtration rate and significantly higher resting B P than those with normal and high PRA (standardized for sodium excretion) The findings indicate that plasma renin activity decreased with increase in the severity of the hypertensive disease

Blood pressure and severity of hypertension

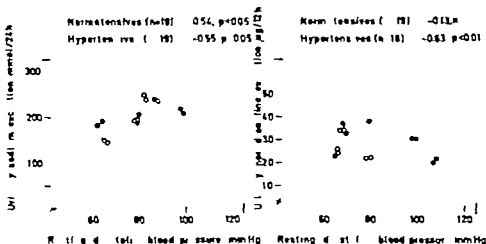
In the hypertensive group a positive correlation was found between resting diastolic B P and the height of the R-wave in lead X of the orthogonal ECG (R_x) while no such relationship was seen among the normotensive subjects (Fig 1) This finding indicates a good relationship between resting diastolic B P and the severity of the hypertensive disease

Figure 1



The relationship between resting diastolic B P and height of the R-wave in lead X of the orthogonal ECG

Figure 2



The rel tionship betwee resting dia toli B P and rinary sodium excretion and urinary noradrenali xcretio

Relationship of blood pre sur to sodium e creti n and sympathetic acti ity

In the normotensive group the r sting di stolic B P was positively correlated to urinary sodium excretion (Fig 2) I the hypert sive group on the oth r hand there was a negative correlation The resting di stoli B P was not ignificantly co related with rinary noradr naline e reti during the day among the normoten ive (Fig 2) but among the hypertensives a negative correlation was found Five hyperte sive subjects had a r sting diastolic B P below 90 mm Hg These subje ts had rinary sodium xcretion levels simila to the patt rn in the normot nsive group and they had high r rinary noradrenaline excretio during the day than the hyperte ive who did not normaliz their B P d ring rest Thi ight indi at that the B P i these five subject was maintained by a high sympathetic tone and that they had not yet developed the patt r with a low urinary sodium xcretion

DISCUSSION

Fig 3 pres ts a hypothesis o the role f the kidney in the developme t of esse ti l hypert sio The hypothesis i an ttempt to i t rp et our results It i known from previous studi that repeated increases of sympatheti discha ge due to for xampl psychologi al stres lead to repeat increases of B P

RESULTS

Plasma renin activity

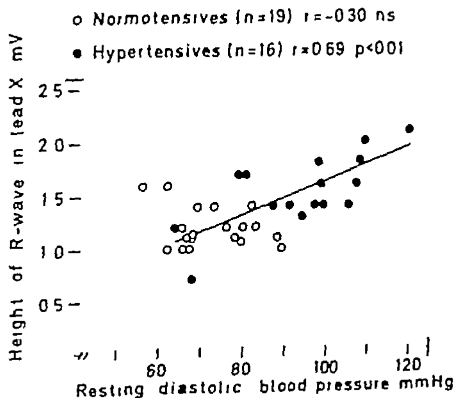
Both in the normotension and in the hypertension group PRA was roughly normally distributed with a slight scewness to the right Mean values were 0 78 and 0 65 respectively the difference being *almost statistically significant* (0 10 > p > 0 05)

Hypertensive subjects with low PRA (standardized for sodium excretion) had a significantly lower glomerular filtration rate and significantly higher resting B P than those with normal and high PRA (standardized for sodium excretion) The findings indicate that *plasma renin activity decreased with increase in the severity of the hypertensive disease*

Blood pressure and severity of hypertension

In the hypertensive group a positive correlation was found between resting diastolic B P and the height of the R-wave in lead X of the orthogonal ECG (R_x) while no such relationship was seen among the normotensive subjects (Fig 1) This finding indicates a good relationship between resting diastolic B P and the severity of the hypertensive disease

Figure 1



The relationship between resting diastolic B P and height of the R-wave in lead X of the orthogonal ECG

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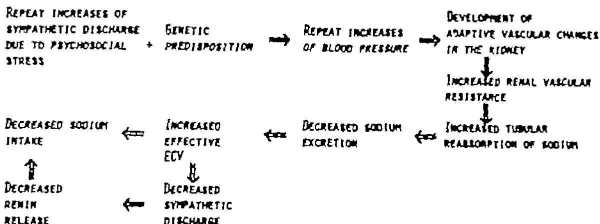
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Figure 3

HYPOTHESIS ON THE ROLE OF THE KIDNEY IN THE DEVELOPMENT OF ESSENTIAL HYPERTENSION



Hypothesis of the role of the kidney in the development of essential hypertension

in genetically predisposed animals This has been shown to lead to development of adaptive vascular changes in the peripheral vascular tree and in the kidney (5) The resulting increased renal vascular resistance may lead to an increased tubular reabsorption of sodium as has been proposed by Kolsters et al (7) This leads to decreased sodium excretion and an increased effective extracellular volume which via receptors on the low-pressure side decreases the sympathetic discharge resulting in a decreased renin release Both the increased effective extracellular volume and the decreased renin release may lead to a decreased sodium appetite (8) which might explain the low sodium excretion in more advanced hypertension

This study was supported by grants from the Swedish Medical Research Council (K73-19x-4131-01 B74-19x-4229-01A) from the Swedish National Association against Heart and Chest Diseases and the Bank of Sweden Tercentenary Fund

Correspondance Göran Berglund M D the Department of Medical I Sahlgren s Hospital 413 45 Göteborg Sweden

MATERIAL AND METHODS

The study hich is n t yet ?p ted will incl de pp o imat ly 120 49 y r-old men ho will b ex ined in 1975 76 Th examined pop lation onsi ts f a ra dom half of ll men born 1926-27 li ing i Göt borg S den (n=3 000) The me re i vited t a sc eening xa inatio including mea urement f blood pressure s-chole te ol tc 76% of those invit d ppe d f r the exa ination The blood pressur wa mea ed i the itting position ft 5 minute rest The diast lic bl d pr e r was egi t red t phase 5 A ubgroup of tho e or en d we a ked if they wo ld b willing to particip t in f the investig tion f th i di nd nal functi The sel ti f the bg up s de ge ding t th di st lic bl od p es nd was a follows 1/30 f ll tho e ith a di tolic blood p ure bel 95 mm Hg 1/15 bet 95 104 mm Hg 1/6 betwe 105 115 mm Hg nd all ith di toli blood pr re xceeding 115 mm Hg All thos ha ing tihyp te i e tr tme t a e luded 90% (n=71) i thi subgroup accept d to pa ti ipat i the study

The inv tigati pr p am we a f llow

Day 1 3 Ambula t c ll ction of uri f r naly es of odi albumine aldost ne and c t hol ine

D y 4 Inulin- nd PAH-cl anc pl a eni ti ity angio-te i II and aldost ne i rl we i tio f p iphar i i leti ith oscill et y and e ous o l i pl thy mog phy The in r f symp th ti nervous ti ity during tilt was a d by me su ing plas a cre lin d pl ma i cti ity

D y 5 Oe ral glucose t l nce t t t t l body pot s ium det r i ati f c l l ti f body ell ma o th ge l ECG pho c di g phy ho c diog phy pl volume (RIHSA) nd measu me t f bl d p s ft r one hou f t in a ound p af oo

Renal function and renovascular resistance in essential hypertension

Susanne Ljungman Marianne Hartford Mattias Aurell
Göran Berglund and John Wikstrand

From the Sections of Preventive Cardiology and Nephrology
Medical Department I and the Department of Clinical Physiology
Sahlgren s Hospital University of Göteborg Göteborg Sweden

In Göteborg in 1975 a study of essential hypertension was started with special reference to cardiac and renal patho physiology The study was a continuation of our previous investigations which showed a decreased urinary excretion of sodium and noradrenaline with increasing severity of hypertension (1-5) Since increased renovascular resistance was one of the possible explanations for these results the continuation of our studies concentrated on determination of the renovascular resistance Several previous studies of the excretion of sodium and noradrenaline in essential hypertension have shown divergent results (6, 9) This can depend on the fact that patients of different ages sexes and various severity of hypertension have been compared By studying individuals of the same age and sex whose blood pressures represent the whole scale from low to high an idea of the hypertensive disease of varying severity can be obtained By using quantitative methods even slight hypertensive organ damage can be detected which may have implications for therapy as well as for prognosis

The aim of this presentation is to briefly present the design of our new study and to acquire some preliminary results concerning the renal function in the group of patients first studied

MATERIAL AND METHODS

The study which is not yet complet d will include pproximately 120 49 year-old men who will be e amined in 1975 76 The e a ined pop l ti n consi t of ra dom half of ll men born 1926-27 living i G t bo g ade (n=3 000) Thes men were invited to a scr eni g xamination incl ding measurement f blo d pr ur -chole te l etc 76% of those invited appear d f r the xa in tio The blood pres r wa meas ed in the itting positio ft 5 minutes rest The diastolic blood pr ss r was regi t red t phas 5 A bgr up of those xaen d wa ask d if they could b illing to particip te i f ther inv stigation f thei c rdi c and r nal f nctio The e l ti f the s bgroup was de garding t the dia t li blood p es nd wa f ll 1/30 of ll tho with diast lic bl d p ssur bel 95 mm Hg 1/15 between 95 104 mm Hg 1/6 betwe 105 115 mm Hg nd ll ith di toli blood p xc ding 115 mm Hg All tho ha ing ntihyp rt ive t atme t ws excl ded 90% (n=71) in this bgroup c pt d to parti ipat i the study

The inv tigatio prog am a f ll

D y 1 3: Ambul nt ll ction f rine fo naly es f odium albumine ldo t one nd t cholamines

D y 4 Inulin- nd PAH l a nc pl sma eni cti ity ngio- t si II nd ldost one i rl sma a insti f p ripho al ci cul tio with oscille t y nd eno c lusi pl thys ography The i t as f ympathetic ous a t ity d ri g tilt a d by ing pla a nored naline a d pl ra nin acti ity

Day 5 P ra l glucos tol nc test tot l body pot sium det rmi tio f al l ti f body ll ma thog al ECG phon rdiogr phy cho-c diog aphy pl a lume (RIHSA) and me e t f blood pr re fte one ho r f re t l sund maf roo

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ten i s (570 ml/min 1 73 m^2 BSA $p < 0\ 05$) but th hematocrite was highe i th hypert nsives which ade the calculated bs l t renal blood flow ppro imately q al (1100 1/min) i the two g oups

R novascular resistance

The renovascul resist nc was calc lated as the ratio between the me t rial p ess r i mm Hg d ring cle and d the abs lute enal blo d flow in ml/min It wa higher in the hypert nsi e g oup (0 104) the in the rmotensi g p (0 081 $p < 0\ 05$) The renova ular esiste o thus inc eas d ith higher blood pres ur

Exc etio f no dr naline

The was a egati o r l ti between the r nov c l ist nce a d the o adre aline r ti n i the hyp ten i gr up (r 0 59 $p < 0\ 05$) and ai il r t dency wa s i the normotensi es (0 40 s)

Sodium or ti n

The c l tion bet n blood p es t rest and the odium tion wa p iti i the no mot siv group (0 58 $p < 0\ 05$) and negati non- ignifi a t tend ncy wa found i th hyp rt nsi e group Th o l ti oeffi i t f the el ti ns b tween reno la esi t nc d dium tion wa e of the s me mag itud e th lati o ffi i t b t een blo d p es r a d s dium xc ti

Thereafter the persons underwent a diagnostic investigation for secondary hypertension at the Out-patient Hypertension Clinic where the blood pressure was measured at three different occasions in the supine position after 5 minutes rest. At clearance the blood pressure was measured every ten minutes during two hours in the semirecumbent position.

RESULTS

Results will be presented from a group of subjects with normal blood pressure and from a group of patients with hypertension. The normotensives were 1/30 of all those with diastolic blood pressure less than 95 mm Hg at screening (n 13). The hypertensives were all those having a diastolic blood pressure over 115 mm Hg at screening and as well over 104 mm Hg as the mean diastolic pressure of three readings at the Out-patient Hypertension Clinic (n 11).

Blood pressure

The arithmetical means of the blood pressures at screening, during clearance and after one hour's rest in the normotensive group were 133/85, 115/71 and 113/65 mm Hg, respectively, and in the hypertensive group 184/124, 151/94 and 150/91 mm Hg. The blood pressure during clearance was almost as low as after one hour's rest and can thus be regarded as basal.

Renal function

There was no significant difference concerning inulin clearance between the two groups. PAH-clearance was significantly lower in the hypertensives (520 ml/min 1.73 m^2 8 4) than in the normo

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DISCUSSION

What is the cause of the increased renovascular resistance in essential hypertension? In earlier studies it has been proposed that the increased renovascular resistance is caused by a raised activity of the sympathetic nervous system (7). In that case the excretion of noradrenaline should increase with raised renovascular resistance. In contradiction to this theory we found a negative correlation between blood pressure, and excretion of noradrenaline and also between renovascular resistance and excretion of noradrenaline.

Our preliminary interpretation of the relation between the investigated variables is as follows:

With increasing blood pressure the renal blood flow is chiefly unchanged while the renovascular resistance increases. Parallel to the increasing renovascular resistance the excretion of noradrenaline and probably also the excretion of sodium decreases in the hypertensives. The raised renovascular resistance cannot be explained by increased sympathetic activity so there must be some other mechanism, probably adaptive structural vascular changes in the renal vascular bed (8), that may lead to a deterioration of the renal ability to excrete sodium. Through a yet unknown mechanism the hypertensives seem to reduce their salt intake with increasing severity of hypertension. The sodium balance could therefore be a factor of importance determining the blood pressure level.

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Intrarenal pressure and sodium excretion in essential hypertension

Y Willassen and J Ofstad,
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Experimental studies in the laboratory animal indicate that the peritubular hydrostatic and oncotic forces influence the renal tubular transport of sodium Whether these factors are operative in the regulation of sodium excretion in the human kidney in health and disease is not known
According to Guyton individuals with essential hypertension have a diminished renal capability for sodium excretion leading to sodium and water retention and increased blood pressure The resulting hypertension is thought to act as a feedback mechanism bringing the sodium balance back to normal by increasing the renal salt and water excretion This so-called pressure natriuresis could be mediated by increased hydrostatic pressure in the peritubular capillaries which also probably could explain the exaggerated natriuretic response to saline infusion in individuals with essential hypertension
We have studied intrarenal pressure and sodium excretion in 9 patients (mean age 40 years) with uncomplicated essential hypertension (EHT) of moderate degree and in 12 health normotensive ( ) individuals (mean age 25 years) Control studies (C) were ... the fasting, hydropenic state in the morning followed by observat... during sustained expansion (E) with 0 9% NaCl given intravenously, increasing the body weight ...
Glomerular filtration rate (GFR) renal blood flo ( ) and filtration fraction (FF) was calculated using clearance for inulin and PAH and extraction ratios for PAH

Intrarenal venous pressure (IRVP), considered a reliable index of peritubular hydrostatic pressure was measured with a catheter wedged in the subarcuate region of an interlobar vein and mean arterial pressure (AP) was measured directly in the brachial artery

The results are given in the tables Absolute values for diuresis (V) and natriuresis (U_{Na} V) are given as well as the per cent increases from control values during sustained expansion (ΔV and ΔU_{Na} V) Values are means ± 1 SEM.

ǂ : Significantly different from control value in same group (p< 0 05)

§ : Significantly different from corresponding value in NT (p< 0 05)

		AP	IRVP	GFR	RBF	FF
		mmHg	mmHg	ml/min	ml/min	%
NT	C	85±2	25 2±1 2	128±4	1356±70	19 7±0 9
	E	84±2	25 4±1 2	133±6	1453±104	18 1±0 8
EHT	C	102±3 §	25 6±1 6	120±6	1116±61 §	23 1±1 1 §
	E	103±3 §	25 8±1 6	144±10	1345±86	21 0±1 0 §

		V	ΔV	U_{Na} V	ΔU_{Na} V
		ml/min	%	µeq/min	%
NT	C	1 1±0 1	277±46	164±13	364±58
	E	5 9±0 4		709±67	
EHT	C	0 9±0 1	651±173 §	137±20	839±147 §
	E	7 0±1 8		1215±259 §	

IRVP did not change significantly during saline expansion in NT and EHT During the early phase of volume expansion small transient

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IRVP did not change significantly during saline expansion in NT and EH. During the early phase of volume expansion small transient

increases in intrarenal pressure were observed in a few individuals both in NT and EHT During sustained expansion the pressures gradually fell towards control values in these cases Evidently natriuresis of the magnitude observed in this study can be induced and sustained by saline without any increase in IRVP and, by inference, peritubular capillary pressure

The hypertensive group clearly exhibited exaggerated diuresis and natriuresis during volume expansion As the intrarenal pressure did not differ from the normotensive group, increased intrarenal pressure can be considered neither an essential prerequisite for the exaggerated natriuresis during volume expansion in essential hypertension nor a mediator of the pressure natriuresis according to Guyton's hypothesis

Considering the age difference between the groups in this study, no correlation could be found between age and IIVP in control condition in 14 patients with EHT aged 24-55 years (mean 40 6)

SYNTHESIS OF RENIN INHIBITORS

A REVIEW

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Summary

This short review describes how competitive inhibitors were synthesized and how the solubility was increased in order to make them effective at physiological pH Their use for in vitro and in vivo inhibition of renin is discuss d and their use for purification of renin demonstrated

---

The basis for our investigation was the work of Skeggs et al [1] who determined the amino acid sequence around the cleavage site of renin-substrate and also determined the smallest peptide with the native amino-acid sequence which could be cleaved by renin at a reasonable rate This peptide was an octapeptide and had the sequence

Fig 1

Asp-Arg-Val-Tyr Ile-His Pro-Phe Hi -Leu-Leu-Val-Tyr-Ser
(Tetradecapeptide)

Asp-Arg-Val-Tyr-Ile His Pro-Phe-His-Leu
(Angiotensin-I)

His-Pro-Phe-His Leu Leu-Val-Tyr
(Octapeptide)

His Pro-Phe-His Leu-D-Leu-Val-Tyr
(D-Leu⁴-octapeptide)

His Pro-Phe-His Leu Leu Val-Tyr An even shorter peptide Leu Leu-Val Tyrosinol wa synthesiz d by Kokubo et al [2] and was shown to be a competitive although weak inhibitor for renin We first re synthe ized the octapeptide and confirmed that it w s a substrate for renin and could be cleaved at the Leu-Leu bond [3] This peptide with the native amino-acid sequence was not only cleaved by renin but also acted as a competitive substrate when human renin reacted with the tetradecapeptide The tetradecapeptide Asp-Arg Val-Tyr-Ile His Pro-Phe His Leu Leu Val-Tyr Ser is a larger peptide with the native sequence which when cleaved by renin forms angiotensin I Asp-Arg Val-Tyr-Ile-His Pro-Phe-His Leu [1] The octapeptide acted therefore as a competitive inhibitor since its cleavage product was too short to be angiotensin-I o to have immunological similaritie with angiotensin I How ver the octapeptide was cleaved

increases in intrarenal pressure were observed in a few individuals both in NT and EHT During sustained expansion the pressures gradually fell towards control values in these cases Evidently natriuresis of the magnitude observed in this study can be induced and sustained by saline without any increase in IRVP and, by inference, peritubular capillary pressure

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Considering the age difference between the groups in this study, no correlation could be found between age and IRVP in control condition in 14 patients with EHT aged 24-55 years (mean 40 6)

at pH 5 5 but was without effect at pH 7 5 This inhibitor was attached to a solid support sepharose 4B by a covalent bond The support was packed as a column and an impure hog r nin sample was applied at pH 5 5 At this pH renin was bound tightly to the inhibitor and therefore retained in the column (whereas other proteins were not) when the column wa washed with a buffer at pH 5 5 After the impurities had been removed in this way renin could be liberated again by increasing pH to pH 7 5 at which pH the inhibitor was no longer an inhibitor for renin Hog renin was in this way purified 200-fold from an already purified sample [6] and we now have indications from N-terminal amino acid analysi that the renin is more than 33% pure

It i our hope that further research along these lines will provide us with effective inhibitors for human renin which can be used in the clinic

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by renin A much more efficient inhibitor would be a peptide with affinity for the enzyme site of renin but which was not cleaved and therefore more stable

The first approach was to substitute some of the amino acids in the octapeptide with D-amino acids instead of the naturally occurring L-amino acids (Fig 1) This has been shown for other enzymes to block the cleavage without impairing the binding to the enzymatic site of the enzyme The D-Leu[4]-octapeptide was a true competitive inhibitor judged by weighted Lineweaver-Burk plots and was not cleaved by renin The use of this inhibitor and a series of related inhibitors were however restricted to in vitro studies because they were not very soluble at physiological pH

A study was then undertaken to increase the solubility [4] The best method involved a prolongation of the peptide chain with from one to five proline molecules added to the N-terminal of the peptide

Fig 2 Human renin inhibitors, pH 7 5

	1	2	3	4	5	6	7	8	K_i (µM)	Solubility (µM)
	His	Pro	Phe	His	Leu	Leu	Val	Tyr	-	161
Pro									42	324
						D-Leu			-	137
Pro						D-Leu			-	41
Pro-Pro-Pro						D-Leu			-	365
Pro						Tyr			12	303
Pro						Phe			4	412
Pro					Phe	Phe			1	100

(Fig 2) Proline addition also increased the solubility of D-Leu[4] octapeptide but to our surprise this peptide which was the best inhibitor at pH 5 5 was completely without effect at physiological pH Fortunately other substitutions showed inhibitory properties at physiological pH This is especially true when hydrophobic residues such as tyrosine and phenylalanine are substituted for leucine in position 6 The best inhibitor so far is a double substitution with a phenylalanine substituted for each of the two leucines and solubilized by an N-terminal proline (Fig 2) This peptide is a true and effective competitive inhibitor for human plasma as substrate In both cases the K_i-value is 1 µM (Fig 2) This is an inhibitor constant of about the same size as the K_m value (0 4 µM) for the reaction between human renin and its protein substrate in plasma and the plasma renin activity can be completely blocked

This inhibitor was therefore expected to be effective in vivo but it was without effect in rats This lack of effect was shown to be due to a species specificity [5] The inhibitors were modelled for human renin and were almost without effect for renin from other species Studies with monkeys are now under way This species specificity makes it likely that very specific inhibitors for renin can be synthesized but that they will have different effect in different species

Finally a successful use of the inhibitors will be mentioned As mentioned above the D-Leu[4]-octapeptide was an effective inhibitor

at pH 5 5 but was without effect at pH 7 5 Thi inhibitor was attached to a solid support sepharose 4B by a covalent bond The support was packed as a column and an impure hog renin sample was applied at pH 5 5 At this pH renin was bound tightly to the inhibitor and therefore retained in the column (whereas other proteins were not) when the column was washed with a buffer at pH 5 5 After the impurities had been removed in this way renin could be liberated again by increasing pH to pH 7 5 at which pH the inhibitor was no longer an inhibitor for renin Hog renin was in this way purified 200-fold from an already purified sample [6] and we now have indications from N-terminal amino acid analysis that the renin is more than 33% pure

It is our hope that further research along these lines will provide us with effective inhibitors for human renin which can be used in the clinic

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EFFECT OF BETA$_1$ RECEPTOR BLOCKADE (METOPROLOL SELOKEN^R) ON BLOOD PRESSURE PULSE RATE PLASMA CATECHOLAMINES RENIN ACTIVITY AND URINARY ALDOSTERONE

B G Hansson and B Hökfelt
Department of Endocrinology, Malmö General Hospital, Malmö, Sweden

Nine patients with moderate hyperten ion were studied under defined metabolic conditions before treatment after one month on placebo and after three months treatment with metoprolol 50 150 mg three times daily Eight patients were considered to have essential hyp r tension one of whom showing remarkably low urinary aldosterone On had polycystic renal disease Plasma metoprolol concentrations were determined repeatedly samples always been taken one hour after the last proceeding metoprolol intake

Placebo had no effect on any of the parameters studied

During hospitalization blood pressure before treatment was 147/103 + 6/6 (mean $\pm$ SD) in supine position and 139/102 + 10/7 in upright position Treatment with metoprolol in the maximal dosages used resulted in a significant decrease in systolic and diastolic blood pressure to 129/87 + 10/9 in supine and 118/87 + 11/9 in upright position ($P < 0$ 001) Before treatment pulse rate was 70 + 8 in supine and 88 + 10 in upright position and during metoprolol 57 + 9 in supine and 69 + 12 in upright position ($P < 0$ 001) The dose needed for reduction of blood pressure varied considerably from one patient to the other and was not correlated to pre treatment values for pulse rate plasma catecholamines or renin activity

During metoprolol all patients showed the same maximal working capacity as compared to before medication but a reduction of the response in pulse rate (P < 0 001) and systolic blood pressure (P < 0 02) to maximal work occurred Both before and during metoprolol the plasma levels of noradrenaline showed great intra and interindividual variations in supine and upright posture in spite of care having been taken to collect the blood samples under identical conditions from day to day Treatment with metoprolol lead to no significant changes in plasma catecholamine content neither in supine nor upright position (P 0 05) There was a good correlation between the plasma catecholamine levels and the urinary excretion of noradrenaline (r 0 72 P < 0 005) and adrenaline (r 0 56 P < 0 05)

In order to explore the effect of metoprolol on plasma catecholamines and renin activity during work these parameters were followed in connection with an individualized standardized submaximal work test for six minutes the pulse rate in steady state being 130 per minute Before treatment submaximal work caused a marked increase in plasma noradrenaline (80 2 46 8 ng/100 ml) This noradrenaline response was further increased under medication with metoprolol (165 1 122 9 ng/100 ml P 0 02) Plasma adrenaline showed no significant changes following work neither before nor during treatment with metoprolol (P > 0 05)

Before treatment two patients showed rather low plasma renin activity four had normal and three patients rather high levels in supine position (193 + 134 ng/100 ml) In all nine patients plasma renin activity increased in response to upright position (464 + 389 ng/100 ml) Metoprolol treatment was followed by a significant reduction in mean plasma renin in supine to 98 + 109 ng/100 ml (P < 0 001) as well as in upright position to 251 286 ng/100 ml (P < 0 025) The reduction in plasma renin activity was correlated to the plasma metoprolol concentrations (r 0 87 P < 0 01) Before treatment all patients

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showed a clear increase in plasma renin activity in response to submaximal work During metoprolol the renin response to work was reduced in four patients and unchanged in four whereas an absolute increase was seen in one

During metoprolol a tendency to a decrease in urinary aldosterone was observed although not statistically significant for the group as a whole (P >0 05) Individual analyses however showed a correlation between the percentage decrease in renin activity and the percentage fall in aldosterone excretion during metoprolol (r = 0 86 P <0 01) demonstrating that the more pronounced the decrease in plasma renin activity the more pronounced decrease in the aldosterone excretion

Reference

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Plasma renin activity and blood pressure during long t rm treatment with propranolol and diuretic

I Nielsen, P Steiness and B Hesse

From Medical Departm nt B Rigshospitalet and Department of Pharmacology University of Copenhagen Denmark

Abstract 19 primary hypertensive patients were treated in random succession with diuretic propranolol and the combination of both It was found that the diur tic gave a sustained increase in las a renin activity (PRA) There was a negative correlation between PRA level attained and blood pressure reduct on Propranolol treatment gave a sustained reduction of PRA There was no correlation b tween PRA reduction and blood p ess re reduction When diuretic was combined with propranolol PRA was in average at control level and there was no correlation between the PRA reduction from diuretic value to combination value and the corresponding additional blood pressure fall It is concluded that propranolol t atment does not transform patients to low renin hypertensives specifically sensitive to diuretic treatment

Plasma renin as a patogenic factor in prim ry hypertension and a par ment r worth measuring to hose herapy has been a controversial subj ct since Buhl r et al (2) found that high renin hyp rtension responded best to propranolol t eatment and there s si nificant correlati n between fall in plasma renin activity and f ll in blood pressure Only very few inv stigators have b en able to reproduce these findin s That pl sma renin activity indeed is a blood pressure deter inant was s pported y Huber et al (6) who found that normot nsives only l ing s lt depletion (with onsequently high enin) respond d to inhibitor to converting enzyme (impeding angiot nsin II production) with blood pressure fall during orthostasis The observations by Douglas et al (4) that low renin hypertensives re ponded best to di retic tr tment has aided evidence to the contention

The object of the present study w s to establish whether pr pranolol ould suppr ss plasm r nin activity du ing diu etic t atment Furth r to valu te wh th r a pos ible renin s ppression had ade th pati nt a low renin hyp rten ive with a pronounced additional blood pr ssu fall d ring combined propranolol and

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diuretic treatment

MATERIAL

Nineteen patients, 4 women and 15 men, (average age 45 years, range 23 - 62) with primary hypertension according to WHO criteria 1 and 2 were examined None of the patients had previously received antihypertensive treatment The patients were seen 3-4 times with weekly intervals, the blood pressure readings and plasma renin activity (PRA) (2-4 p m 45 minutes supine) at the two last visits were averaged and formed the control values The range of diastolic pressure was from 110 to 140 and the range of PRA was from 0 5 ng/ml/h to 12 1 ng/ml/h Then the patients were admitted for routine examination

PROTOCOL

At discharge the patients were randomly started on bendrofluomethazide (Centyl[R]) 10 mg b i d , propranolol (Inderal[R]) 80 mg t i d , or both combined The patients were seen every 3 week in the out-patient clinic, always in the afternoon (2-4 p m) Blood pressure PRA and plasma propranolol concentration were measured at each visit After approximately 3 months the therapy was changed to one of the other two regimens and after further 3 months the last regimen was instituted Five patients with satisfactory blood pressure response to a one-drug regimen were not put on combined therapy but always on the other one-drug regimen ... patients left the trial before completing the third phase for none medical reason Blood pressure PRA and plasma propranolol concentration on the different regimens were calculated as average of all ambulant measurements

METHODS

PRA was measured by radioimmunoassay using the NEF-kit (angiotensin I - J^{125} Haber method coefficient of variation = ± 10%) Normal values mean 2 7 ng/ml/h ± 3 0 (SD) range 1 1 - 8,6 (N = 3) Plasma propranolol concentration was measured by a gaschromatographic method previously described by Walle (9) Student s t-test was used for analysis of significance and as measure of central tendency the standard error of the mean is given in parentesis

RESULTS

Propranolol treatment: PRA fell from 3 5 ng/ml/h (0 8)

(control) to 1 7 ng/ml/h (0 2) ($p < 0\ 05$) Diastolic blood pressure fell from 119 mm Hg (2 0) (control) to 105 mm Hg (3 0) not significant There were neither a significant correlation between decrement of diastolic blood pressure and control PRA ($r = 0\ 42$ $p > 0\ 1$) nor between decrement in diastolic blood pressure and decrement of PRA ($r = 0\ 41$ $p > 0\ 1$) However a significant linear correlation between control PRA and decrement in PRA during treatment was demonstrated ($r = 0\ 97$ $p < 0\ 001$) Plasma propranolol concentration was in the range 40-200 ng/ml but no correlation between propranolol concentration and decrement in diastolic blood pressure ($r = 0\ 19$ $p > 0\ 1$) or between propranolol concentration and decrement in PRA ($r = 0\ 35$ $p > 0\ 1$) was found

Diuretic treatment: PRA rose from 3 5 ng/ml/h (0 8) (control) to 6 4 ng/ml/h (1 3) ($p < 0\ 05$) Diastolic blood pressure fell from 119 mm Hg (2 0) (control) to 109 mm Hg (3 0) ($p < 0\ 001$) There was a significant negative correlation both between control PRA and decrement in diastolic blood pressure ($r = -0\ 57$ $p < 0\ 001$) and between PRA during diuretic treatment and decrement in diastolic blood pressure ($r = -0\ 62$ $p < 0\ 001$) The increments of PRA from control was not correlated to the decrement in diastolic blood pressure ($r = 0\ 28$ $p > 0\ 1$)

Combined treatment: PRA was unchanged from control value 3 5 ng/ml/h (1 1) Diastolic blood pressure fell from 119 mm Hg (2 0) to 99 mm Hg (3 0) ($p < 0\ 001$) There was no correlation between PRA on diuretic treatment and decrement in diastolic blood pressure on combined treatment ($r = 0\ 12$ $p > 0\ 1$) There was no significant correlation between decrement in PRA from diuretic treatment to combined treatment and the corresponding diastolic blood pressure decrement ($r = 0\ 22$ p 0 1) However there was a significant correlation between PRA on diuretic treatment and decrement in PRA during combined treatment ($r = 0\ 67$ $p = 0\ 05$)

DISCUSSION Like the majority of other investigators were were unable to find a correlation between initial PRA or PRA reduction and blood pressure reduction during chronic propranolol treatment (1 3 7 10) In contrast to the findings of Leonetti et al (8) we found no correlation between plasma propranolol concentration and blood pressure decrease - but it must be emphasized that the former study was not a chronic treatment In diuretic treatment

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MONOAMINE METABOLITES IN CEREBROSPINAL FLUID DURING TREATMENT WITH CLONIDINE OR ALPRENOLOL+

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An antihypertensive drug with probable central mechanism of action (clonidine) and an adrenergic beta-receptor blocking drug (alprenolol) have been investigated regarding their effects on the concentrations of the major metabolites of noradrenaline (4-hydroxy-3-methoxyphenyl glycol HMPG) serotonin (5-hydroxyindoleacetic acid 5-HIAA) and dopamine (homovanillic acid HVA) in cerebrospinal fluid (CSF)

Five of six patients treated with clonidine (doses 450-900 µg/day) showed a significant lowering of their blood pressures together with a decrease of HMPG in CSF In the sixth patient there was no effect on blood pressure or HMPG

Four patients treated with alprenolol (doses 600 mg daily) showed decrease in blood pressure and heart rate (both at rest and maximal workload) but no changes in HMPG in CSF

HVA and 5-HIAA levels in CSF were not affected by treatment with either of these two drugs

Our observations in man are consistent with the previous findings in the rat and the monkey that clonidine lowers HMPG-levels in CSF presumably as a result of feed-back inhibition of noradrenaline synthesis caused by its adreno ceptorstimulating activity

+The full paper is in press in Eur J clin Pharmacol

CENTRAL HYPOTENSIVE EFFECTS OF ANGIOTENSIN II IN THE RAT

Heikki Karppanen, Tapani Ukkonen, Ilari Paakkari and Pirkko Paakkari

From the Department of Pharmacology, University of Oulu, SF-90220 Oulu 22, Finland

Abstract. *The floor of the fourth cerebral ventricle of urethane-anaesthetized rats was exposed through the occipital foramen. Angiotensin II (10–1000 ng) applied onto the surface of the area postrema, induced rapid lowering of the blood pressure. Pretreatment of the rats with reserpine abolished the hypotensive response to angiotensin II. It is concluded that the local application of angiotensin II induced in the vicinity of the area postrema release of some biogenic amine, which has an inhibitory effect on the cardiovascular centres.*

The area postrema, which lies outside the blood-brain barrier and protrudes into the fourth cerebral ventricle, mediates the central cardiovascular response to angiotensin II in the dog (2). The ablation of the region of the area postrema induces arterial hypertension in rats (4). In the present work the blood pressure effects of angiotensin II, applied onto the surface of the area postrema, were studied in urethane-anaesthetized rats.

MATERIAL AND METHODS

Male Sprague–Dawley rats (250–300 g) were anaesthetized with urethane (1.5 g/kg p.). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously. The mean blood pressure was measured directly from the left femoral artery by means of pressure transducer (Harvard apparatus 377). The rats were placed in stereotaxic instrument. The floor of the fourth cerebral ventricle was exposed via the foramen occipitale. The drug solutions were administered onto the surface of the area postrema in volume of 4 µl by using the stereotaxic instrument. Angiotensin II (Hypertensin, Ciba) was dissolved in 0.9 % (w/v) NaCl.

RESULTS

The application of angiotensin II (10–1000 ng) onto the surface of the area postrema induced lowering of the blood pressure (Fig. 1). This effect was evident within 1 min. The hypotensive effect of 10 ng of angiotensin II lasted for 20 min, and that of 100 or 1000 ng for at least 30 min.

In order to study whether endogenous biogenic amines are involved with the paradoxical hypotensive effect of angiotensin II, rats were reserpinized. Reserpine (2.5 mg/kg c.) was administered on three consecutive days in order to deplete the endogenous stores of biogenic amines. The experiments were started 3–4 hours after the last injection of reserpine. The mean arterial pressure in the reserpinized rats was 92 5 (s.e.) mm Hg, whereas the mean arterial pressure in the control rats was 108 ± 6 (s.) mm Hg.

In the reserpinized rats the hypotensive response to angiotensin II was completely abolished (Fig. 2). After the highest dose (1000 ng) slight hypertensive effect was observed.

MONOAMINE METABOLITES IN CEREBROSPINAL FLUID DURING TREATMENT WITH CLONIDINE OR ALPRENOLOL+

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Depts of Medicine and Clinical Pharmacology Huddinge
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An antihypertensive drug with probable central mechanism of action (clonidine) and an adrenergic beta-receptor blocking drug (alprenolol) have been investigated regarding their effects on the concentrations of the major metabolites of noradrenaline (4-hydroxy-3-methoxyphenyl glycol HMPG) serotonin (5-hydroxyindoleacetic acid 5-HIAA) and dopamine (homovanillic acid HVA) in cerebrospinal fluid (CSF)

Five of six patients treated with clonidine (doses 450-900 µg/day) showed a significant lowering of their blood pressures together with a decrease of HMPG in CSF In the sixth patient there was no effect on blood pressure or HMPG

Four patients treated with alprenolol (doses 600 mg daily) showed decrease in blood pressure and heart rate (both at rest and maximal workload) but no changes in HMPG in CSF

HVA and 5-HIAA levels in CSF were not affected by treatment with either of these two drugs

Our observations in man are consistent with the previous findings in the rat and the monkey that clonidine lowers HMPG-levels in CSF presumably as a result of feed-back inhibition of noradrenaline synthesis caused by its adrenoceptorstimulating activity

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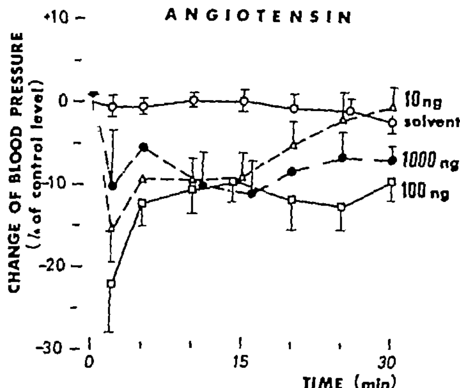


Fig. 1. The dose-response relationship of various doses of angiotensin II. Angiotensin II was applied onto the surface of the area postrema in a volume of 4 µl. Vertical bars indicate s.e.m. The number of rats in each group was 5–6.

DISCUSSION

The authors are not aware of previous studies reporting a hypotensive response to angiotensin II under any circumstances. It should be emphasized that in the present work angiotensin II was confined to a very limited area in the medulla oblongata. Therefore the peripheral hypertensive effects as well as the effects mediated via other angiotensin-sensitive structures in the brain (1) were excluded. The absence of the hypotensive effect in the reserpinized rats indicates that endogenous biogenic amines are involved with this effect of angiotensin II. The lower blood pressure level in the reserpinized rats does not explain the absence of the hypotensive response since an application of clonidine still exerted a hypotensive effect. Although the total amount of angiotensin II was small, the local concentration in the region of the area postrema could have been rather high. It is known that higher concentrations of angiotensin II may induce release of noradrenaline from sympathetic nerve endings (3). Therefore, the hypotensive effect observed in the present study may represent a pharmacological rather than a physiological effect of angiotensin II.

In conclusion, the hypotensive effect of angiotensin II in the region of the area postrema appears to be due to a release of an endogenous biogenic amine which exerts an inhibitory effect on cardiovascular centres.

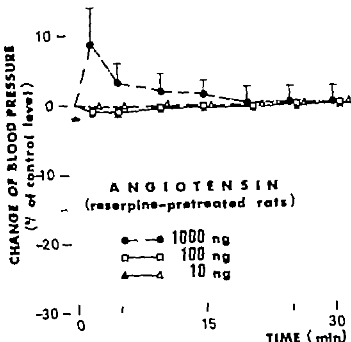


Fig. 2. Time-response relationship of various doses of angiotensin II in reserpinized rats. Reserpine (2.5 mg/kg s.c.) was given on three consecutive days before the experiment.
For further explanation, see the legend of Fig. 1.

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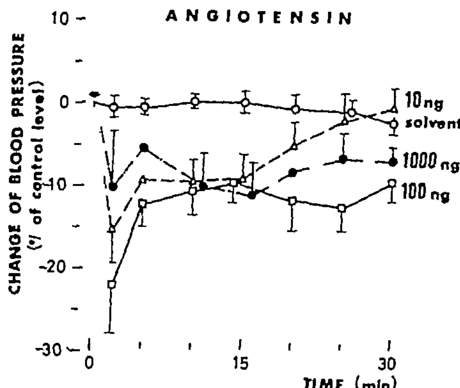


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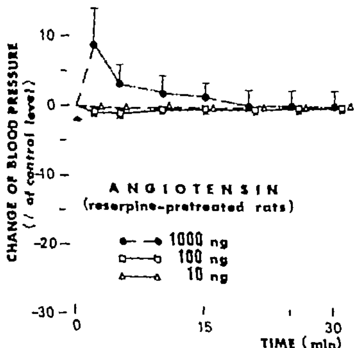


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SITE AND MODE OF ACTION OF CLONIDINE IN THE CENTRAL NERVOUS SYSTEM

Ilari Paakkari, Heikki Karppanen and Pirkko Paakkari

From the Department of Pharmacology University of Oulu, SF 90220 Oulu 22, Finland

Abstract *In urethane-anaesthetized rats clonidine was administered intravenously (i. v.) intracerebroventricularly (i.c. v.) or onto the surface of the area postrema which protrudes into the fourth cerebral ventricle. In each instance clonidine induced a dose-dependent lowering of the blood pressure. The region of the area postrema appears to be the most sensitive site for the action of clonidine so far studied. In order to obtain similar blood pressure effects, approximately 8 times higher amounts were needed i.c.v. and about 80 times higher amounts i.v. than onto the surface of the area postrema.*

A pretreatment of the rats with the specific histamine H_2-receptor blocking drug, metiamide (4.5 µmoles/rat i.c.v.) shifted the dose-response curve of clonidine (i.c.v.) to the right.

The results suggest that clonidine exerts its hypotensive effect in the rat via a stimulation of histamine H -receptors in, or in the vicinity of the area postrema.

Clonidine is thought to exert its antihypertensive effect by acting on the central nervous system (for review see 16) It has been suggested that its main site of action is the medulla (11–13). Bousquet and Guertzenstein have proposed that the site of action of clonidine is on the ventral surface of the medulla (4,5). However a hypothalamic action of clonidine has also been demonstrated (8, 14 15) Flach has reported that centrally administered clonidine has an emetic effect in conscious cats (7) The emetic effect of several drugs is mediated via the area postrema (see 3). The ablation of the region of the area postrema induces a marked hypertension in the rat (17).

In the present work the possible involvement of the region of the area postrema with the hypotensive effect of clonidine was studied. Furthermore our previous proposition that the hypotensive effect of clonidine may be due to a stimulation of central histamine H_2-receptors (9), was further studied.

MATERIALS AND METHODS

Male Sprague–Dawley rats (250–300 g) were anaesthetized with urethane (1.5 g/kg i.v.). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously The mean blood pressure was measured directly from the left femoral artery by means of a pressure transducer (Harvard Apparatus 377). The rats were placed in the stereotaxic instrument and an injection needle was introduced into the lateral cerebral ventricle A polyethylene catheter filled with the solution to be infused, was attached to the needle The desired amount of the solution was allowed to flow by virtue of hydrostatic pressure. At the end of each experiment methylene blue was injected and the proper position of the needle in the cerebral ventricle was ascertained The floor of the fourth cerebral ventricle was exposed via the foramen occipitale The drug solutions were administered onto the surface of the area postrema in a volume of 4 µl by using the stereotaxic instrument

Clonidine hydrochloride (Boehringer Ingelheim) was dissolved in 0.9 % (w/v) NaCl Metiamide (Smith, Kline and French Laboratories Ltd) was dissolved in 0.1 N HCl, and the pH was adjusted t 6 by using 0.1 N NaOH.

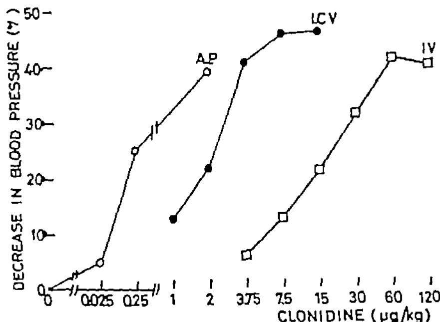


Fig. 1. Influence of the route of administration on the hypotensive effect of clonidine. Clonidine was administered onto the surface of the area postrema (A.P.), intracerebroventricularly (I.C.V.) or intravenously (I.V.). Each point represents the mean from 5–8 experiments.

RESULTS

The administration of clonidine intravenously (i.v.), intracerebroventricularly (i.c.v.) or onto the surface of the area postrema (a.p.) induced in each instance dose-related lowering on the blood pressure (Fig. 1). However about 10 times higher amounts of clonidine were needed than i.c.v. in order to obtain equal effects on blood pressure. The region of the area postrema was extremely sensitive to the effect of clonidine. Only approximately 0.25 µg/kg of clonidine was needed to achieve a 25 % decrease in the level of blood pressure. About 80 times more 20 µg/kg, was needed in order to obtain comparable effect on the blood pressure. A pretreatment of the rats with metiamide (4.5 µmoles/rat i.c.v.), which is specific antagonist of histamine H_2-receptors (2), shifted the dose-response curve of clonidine to the right (Fig. 2). The shift appeared to be parallel, without any change in the maximal response to high doses of clonidine.

DISCUSSION

The present results confirm the previous findings that the site of action of clonidine is in the central nervous system (11, 12, 13, 16). The region of the area postrema proved to be extremely sensitive to the effect of clonidine. In comparison to the ventral surface of the brain stem (5), 80–1000 times lower amounts of clonidine were required on the area postrema to obtain blood pressure lowering effect. On the basis of the present information the region of the area postrema appears to

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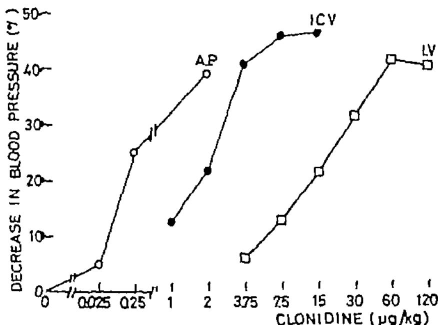


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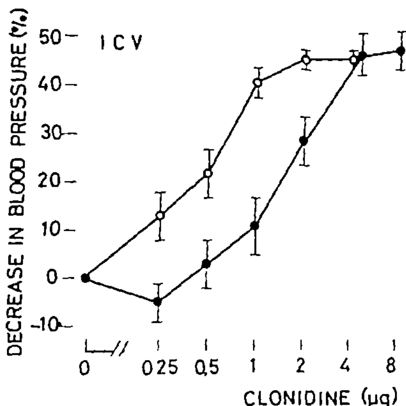


Fig. 2. Lowering of blood pressure after intracerebroventricular (I.C.V.) administration of clonidine. Cumulative doses of clonidine were administered to non-pretreated (o—o) and to metiamide-pretreated (●—●) rats at 20-min intervals.
Metiamide (4.3 µmoles/rat i.c.v.) was given 30 min before the start of clonidine administration. Means ± s.e.m. from 6–7 experiments are shown.

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In conclusion, in the urethane-anaesthetized rats the hypotensive effect of clonidine seems to be mediated by the region of the area postrema. This effect appears to be due to a stimulation of histamine H_2-receptors.

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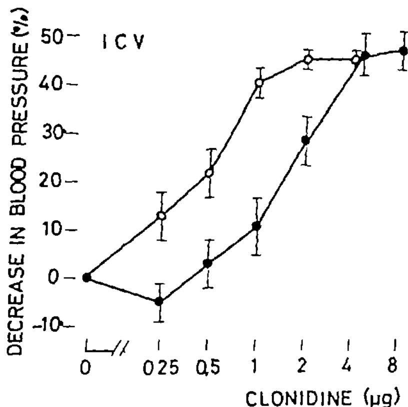


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CLINICAL AND HEMODYNAMIC EFFECTS OF HIGH DOSES OF SLOW RELEASE FUROSEMIDE IN ARTERIAL HYPERTENSION A DOUBLE-BLIND CROSS-OVER STUDY WITH CYCLOPENTHIAZIDE

by

A R Krogsgaard, J Trap-Jensen, O Hartling & T Lysbo Svendsen

Furosemide in daily doses of 40-80 mg has an antihypertensive effect comparabel to thiazides (Wertheimer et al 1971)

The dose-response curve for the diuretic effect of furosemide does not show the same plateau for the maximum response as seen in thiazides High doses of furosemide may therefore have a more pronounced antihypertensive effect than thiazides (Mroczek et al 1974, Cantarovich et al 1974)

In the present study we have compared furosemide and cyclopenthiazide in a double blind cross-over study During an eight week period the daily dose of the drug was increased every second week rom initially 6o mg of furosemide or 0 5 mg of cyclopenthiazide to 360 mg or 3 mg of the respective drugs

As the abrupt diuretic effect of furosemide is a great discomfort to many patients we have used a slow release preparation of furosemide

The investigations have been performed in out-patients with control of blood pressure urinary volume urinary excretion of sodium and potassium serum electrolytes and body weight

Cardiac output and plasma volume were measured before and after eight weeks of treatment with one of the drugs at a time when the highest dose was given Cardiac output was measured using the indicator dilution technique at rest supine and after five min in the standing position

RESULTS

This report is *preliminary* and *the material comprises* the first 8 patients

Blood pressure fell in all periods both supine and erect (Fig 1) Increasing doses of cyclopenthiazide up to 2 mg daily induced an increasing response Concerning furosemide a dose-response relation was not obvious There was no difference between the maximum antihypertensive effect of furosemide and cyclopenthiazide

Body weight decreased slightly but significantly during the maximum dose period For cyclopenthiazide 0 8 kg and for furosemide 0 9 kg

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Urinary volume increased moderately during the maximum dose period It is of interest, that the highest volume measured during furosemide was only 3950 ml per 24 hrs

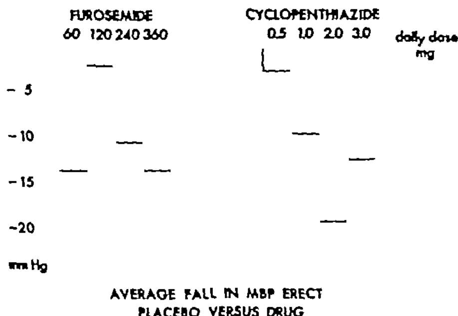
Serum potassium decreased significantly with both drugs compared to placebo

Cardiac output During furosemide treatment cardiac output at rest supine and in the standing position was essentially unchanged as compared to the values obtained in the untreated state During treatment with cyclopenthiazide cardiac output at rest supine decreased in all patients from a mean value of 6 38 l/min before, to 5 40 l/min during treatment In the standing position the fall in cardiac output was less pronounced, the mean values being 4 87 l/min before and 4 22 l/min during treatment

Plasma volume was essentially unchanged during treatment with furosemide, but decreased significantly during cyclopenthiazide treatment as compared to the control values

Summarizing Furosemide in doses up to 360 mg daily may not have a more pronounced antihypertensive effect than cyclopenthiazide in a dose of 2 mg daily Cardiac output and plasma volume were essentially unchanged during treatment with furosemide but decreased during cyclopenthiazide treatment

It must be emphasized that the present report is preliminary and definite conclusions cannot be made until the number of patients has been increased



AVERAGE FALL IN MBP ERECT
PLACEBO VERSUS DRUG

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The pati nts were followed up to the end of the year 1973 The survival curves were calculated by the d crement method (life tables) d scribed by Bonnevi et al (1) Aim was taken from patient ge 65 years and less

RESULTS

I the period 1957 to 1971 932 patients were diag osed a having arterial hypertension Of these 117 patients or 12 6% of the whol group had grade III and IV reti opathy Women numbered 62 (53%) and men 55 (47%) Th majority had reti opathy classified as grade III or 97 (82 9%) includi g 50 f males (51 5%) Twenty patients had grade IV retinal changes and 12 of these (60%) were women

In the last five y ar period the number of patients with grade III and IV retinopathy had decreased from the two previous five year periods when e pressed in relation to the total number of all grades (I IV) admitted (Table I) (χ^2 (2) 7 82 p 0 975)

The age distribution o admissio is shown i Fig 1 The mea age for the females was 56 y ars and for the male 54 years There were more femal s in the older age gr ups

At the time of diagnosis 70 patients (59 8%) were fou d to hav raised blood urea (> 40 mg%) Eightyfi per cent of th patients ith g ade IV retinopathy and 54 2% of patie ts with grade III retinopathy hade rais d blood rea (T ble II) (χ^2 (1) 6 1 p 0 05)

One hundred patient (85 5%) had an ab ormal ECG but 17 patients (14 5%) had a normal tracing Left entricular hypertrophy was found in 51 rec rds (43 6%) and left a is de iation was found in 16 records (13 7%) i cl ding 9 with o comitant LVH Tw patients had left bundle bra ch block and two had s ide of transmural myocardial infarction Cardiomegaly o X ray wa con idered to be pre ent in 61 pati ts (52 1%) Of thi group nly 54% fulfil led ECG crit ria of LVH Of those who had ormal h art ize chest X ray 30 9% had LVH O admission 19 patie ts (16 2%) had a history of a gi a pectoris 9 patients (7 7%) had had myocardial i f rction and 21 pati ts (17 9%) cerebro asc lar a cide t Some of these events led dir ctly to admission

By the end of 1973 79 (67 5%) of 117 patie ts were dead Post mortem amination was p rformed i 31 (39 2%) The auses of death ar shown in Table III

Most of the de the w re d e to c rebro lar accide ts (28 6%) renal failure (22 8%) and myocardial i fa cti (22 8%) Sudde death was lassified as a ch if death was i stantaneous and u e pe ted o occurred withi 24 ho rs f om the o s t of symtoms Fi patie ts who died a dd ly w r co sidered to ha e sustained acut myoc rdial i farcti n and were l ssified as s ch In two of these th diag sis w confirmed at post mortem and the oth r three had symtoms and ig s gg ti e of a he rt att ck Thr e p ti t who tai ed cerebr a c lar ide t died shortly afterwards and were the efor al o li ted i th group of sudden death On patie t died a res lt of a tihyp te si e treatme t He pre nted with ile s the au e of which ould not be r ealed t lap ratomy This patie t had be taki g Me sin powerful g glio blocki g d g for ome we ks prio t death One patie t committed icide H had b i g R erpi fo sev ral y ars but ppar tly had ot had thi drug for some mo ths prior to his d th Other au e of d ath w e ci oma (4) pulmo ry embo l (3) p eumonia (3) ba t ial ndocarditis (1) po t operative hemorrhag (1) and thma (1)

SEVERE ARTERIAL HYPERTENSION (GRADE III AND IV)

A clinical study on 117 hypertensive patients admitted to the Department of Medicine Landspitalinn Reykjavik 1957 19 1

Thorkell Gudbrandsson and Snorri P Snorrason
From the Department of Medicine Landspitalinn (National Hospital) Reykjavik Iceland

ABSTRACT

The aim of this study was to evaluate the number outcome survival time and causes of death of patients with severe arterial hyper tension who were admitted to the Department of Medicine at Land spitalinn (National Hospital) in Reykjavik during the years 1957 to 1971
During this period 117 patients were found to have severe arterial hypertension (12 6% of all cases of hypertension diagnosed) accor ding to the grading of Keith and Wagener 20 patients with grad IV retinopathy and 97 patients with grade III
The case histories were analysed according to age and se distri bution blood urea electrocardiographic changes heart si e by X ray at the time of diagnosis and final outcome
The survival calculations were done by the decrement method (life tables) and aim taken from patient age 65 years and less
Relatively fewer patients with severe arterial hypertension w re admitted during the last five year period (1967 1971) than during the two previous five year periods The main causes of death w re cerebrovascular accidents (26 6%) myocardial infarctions (22 8%) and renal failure (22 8%)
Approximately 50% of the men and 60% of the women survived fi e years Elevated blood urea values and signs of left ventricular hypertrophy on ECG at the time of diagnosis carried a more si ist r prognosis

No clinical study has as yet been published on the hypertensive population in Iceland It was therefore considered of some inter t to carry out a study reviewing several aspects of severe arterial hypertension as presented by the inpatient population at Landspita linn in Reykjavik

MATERIAL AND METHODS

During the period from 1957 to 1971 117 patients were found to have severe arterial hypertension on admission to the Departm t of Medicine at Landspitalinn The hypertension was considered t be severe if the retinal appearances could be graded as III or I by Keith and Wagener s criteria (5) Throughout the study peri the retinal appearances were assessed by the same ophtalmologi t (K Sveinsson) Patients with secondary hypertension were incl ed in the study Left ventricular hypertrophy (LVH) was considered o be present on the following electrocardiographic criteria T depression ≥ 0 5 mm in leads V_4 V_5 and V_6 together with increa d QRS voltage S in V_1 R in V_5 or V_6 ≥ 35 mm (7)

The patients were followed up to the end of the year 1973
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I the l st fi e y ar period the number of patie ts with grade III and IV retinopathy had decreased from th tw pre i s five year periods wh n e pressed i relation to the total n mb r of all grades (I IV) admitted (Table I) (χ^2 (2) 7 92 p 0 975)

The ag distribution on admission is shown in Fig 1 The mean age for the females w s 56 years and for the males 54 years There were more female in th lder age groups
At the time of diagnosi 70 pati t (59 8%) were fo d to have raised blood urea (≥ 40 mg%) Eightyfiv p r e t f the patients with grade IV reti opathy and 54 2% of patients with grade III retinopathy hade r ised blood rea (Table II) (χ^2 (1) 6 1 p 0 05).

One h ndred patie ts (85 5%) had a abnormal ECG but 17 patients (14 %) had a normal traci g Left ventricular hypert ophy was found i 51 records (43 6%) and left a i d iatio was fo nd i 16 records (13 7%) incl di g 9 with co omit t LVH Two patients had left bundle bra ch block and two had evid n f transmur l myocardial infarcti n Cardiomegaly on X ray wa sid red t be pres nt in 61 patie ts (52 1%) Of this group o ly 54% f lfil led ECG criteria of LVH Of those who had ormal hea t size o chest X ray 30 9% had LVH O admissi 19 patients (16 2%) had a history of ngina pectori 9 p tie t (7 7%) had had myocardi l infarction nd 21 patients (17 9%) cer brovascular ide t Some f thes eve ts led directly to admissio

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Mo t of th de ths were d to c bro ascular accide ts (26 6%) r l failure (22 8%) and myo rdi l i fa cti n (22 8%) Sudden death as cl sified as s ch if death wa i ta t us nd n e pe ted or occurred withi 24 ho rs from th o s t of ymtoms Fi e pati ts who died s dd ly were co sid red t hav su tai ed acute myo ardial infar tio nd were classified a s ch In two of these th diagnosis was nfirmed t post mortem and the other three had ymtoms and sig gg ti of a heart attack Thr e patie ts who s tai ed cerebro a la id t died shortly aft rwards and were th refore also li ted i th group of sudden death One patient died a res lt of tihype t ive treatment H presented with il s the ause of which could ot be revealed t laparatomy This pati t had bee taki g Ma a i a pow rful ga glion blocki g d ug f ome w k prior to death O e patie t committed s icide He had be si g R rpin for se eral years but appare tly had ot had this d ug for some mo ths prior to his d ath Other cau s f d ath were car inoma (4) pulmo ary embo l s (3) pneumonia (3) b t rial endocarditis (1) post perati e hemorrhag (1) and asthm (1)

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Table I

Number of patients with severe hypertension (grade III and IV) as a percentage of the total number of all grades (I IV) of hypertension admitted

Years	Grades III IV number	Grades III IV %	Grade III number	Grade III %	Grade IV number	Grade IV %	Total number of all grades (I IV)
1957 1961	38	15 3	34	13 7	4	1 6	248
1962-1966	45	14 8	33	10 9	12	4 0	302
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Table II

Blood urea at the time of diagnosis and grade of hypertension

	Blood urea mg/100 ml 40	40	Total
Grade III	43	53	96
Grade IV	3	17	20
	46	70	116

Table III

Causes of death

Causes of death	Number of patients	Autopsy
Cerebrovascular accident	21 (26 6%)	7
Renal failure	18 (22 8%)	12
Myocardial infarction	18 (22 8%)	5
Sudden death	11 (13 9%)	2
Heart failure	3 (3 8%)	0
Other causes	16 (20 8%)	7

The results of the calculated survival curves are shown in Fig 2 4 Females survive longer than men but raised blood urea and the presence of left ventricular hypertrophy at the time of diagnosis carried a more sinister prognosis Approximately 40% of these two groups survived five years while about 80% of those who had neither raised blood urea or LVH survived five years

DISCUSSION

The diminishing percentage of patients with severe arterial hypertension in the last five years of the observation period is of interest During this time the hospital admissions system for the Reykjavik area did not change and it seems unlikely therefore that more such patients were admitted elsewhere On the other hand the diagnosis of arterial hypertension was made with increasing frequency in the Department of Medicine during this time Therefore in those last five years there may be a true decline in the severe form of hypertension It is also possible that increased interest and improved treatment for the milder forms of hypertension could play a part This is supported by studies from New Zealand (6) and England (2) which have shown latterly that the incidence of severe hypertension may be declining perhaps partly as a result of earlier treatment
The majority of our patients had raised blood urea at the time of diagnosis and the level seems to correlate with the degree of grading Renal failure is often the outcome of untreated or undertreated severe hypertension This also tends to make prognosis worse (2) as suggested by our study
Relatively few patients (45 6%) fulfilled the ECG criteria of LVH On the other hand an abnormal ECG was found quite often (85 5%) Our strict criteria for the LVH may have missed out a few patients but when present were usually accompanied by a more severe hypertension We have also shown that the presence of LVH seriously affected the prognosis
The three most common causes of death were cerebrovascular accident (28 6%) myocardial infarction (22 8%) and renal failure (22 8%) This is comparable to some other studies (2 3) It is well established that before effective treatment became generally available prognosis was very poor for patients with severe hypertension Keith et al (5) have shown that of untreated patients with grade IV retinopathy only 1% survived five years as compared with 20% of patients with grade III retinopathy When effective treatment became generally available the prognosis of these patients changed dramatically Breckenridge et al found that 34% of grade IV patients and 70% of grade III patients survived five years Comparative results reported by Hood et al (3) were 50% for grade IV and 61% for grade III Our study shows for grade III and IV combined a 50% five year survival for men and 60% for females The better outlook for females with hypertension is well k own b t the reasons are less well established The incidence of hypertension is also smaller in females in the younger age group (50 years) but this gradually increases with age and e ceeds that of males in later years as shown by the Framingham study (4) It is conceivable that the brighter prognosis for women could be partly due to earlier diagnosis
Breckenridge et al (2) reported that raised blood urea at the time of diagnosis carried a shorter survival This is confirmed by our study which also suggests that ECG changes of LVH may carry an equally poor prognosis

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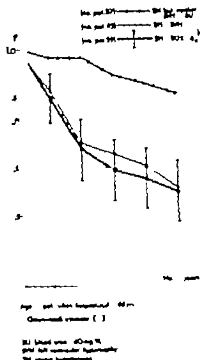


Fig 4 Severe hypertensi (grade III and IV) S r iv l c rves (both e es)

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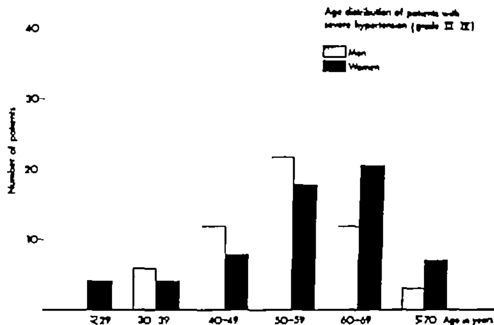


Fig 1: Age distribution of patients with severe hypertension (grade III and IV)

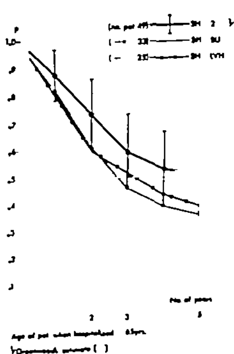


Fig 2 Severe hypertension (grade III and IV) Survival curves (men)

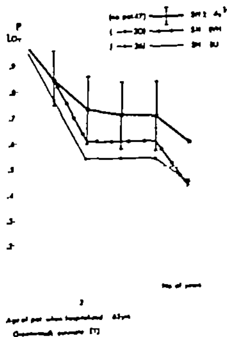


Fig 3 Severe hypertension (grade III and IV) Survival curves (wom)

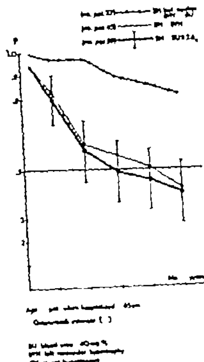


Fig 4 Severe hyperte si n (grade III and IV) Sur ival curves (both s es)

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The treatment of hypertension - an analysis of drug prescription data

G Boethius
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Abstract

This paper describes a study of the degree of continuity in the drug treatment of hypertension An analysis of antihypertensive drugs dispensed to 916 individuals during 1970-74 revealed that 22% of the men and 27% of the women were without treatment for 20-50% of their observation time A closer examination of 46 patients under 60 years of age showed that between 2 and ?0 physicians had been involved in the antihypertensive treatment of the individual patient The time without treatment in this group of unselected patients varied between 0 and 77% of the observation time the degree of discontinuity being insignificantly correlated to the resulting blood pressure level Non-compliance was the cause for discontinuing a drug in at least every fifth case

Introduction

In the long-term treatment of hypertension or any other chronic illness patients may evade treatment and/or control visits for a period of time or they may refuse cooperation completely In other patients in spite of a seemingly good compliance the blood pressure cannot be controlled with any regimen There are a number of reasons for these therapeutic failures (3 5) some of which are quite feasible to overcome (4, 6)

Through the continuous monitoring of prescription drugs dispensed to 1/7 of the individuals in the county of Jämtland Sweden data on these problems are available This paper describes an attempt to elucidate quantitatively the degree of continuity in antihypertensive treatment

Material and methods

The basic methodology for obtaining prescription data is described elsewhere (1) The following data are recorded: the patient s identity number (denoting i e age and sex) the year and week the drug was purchased the dispensing pharmacy, the prescribing doctor the amount dosage and price of the drug and the type of prescription (e g telephone prescription original or repeat prescription)

During the five year period 1970-74 916 persons in the county obtained at least one drug used solely in hypertension (bethanidine guanethidine methyldopa clonidine hydralazine and reserpin) Due to the fact that the indication for the prescribing is not known persons who re eived diuretics and/or betareceptorblocking drugs as the only treatment for hypertensi n during this period are not included The purchase pattern of the 916 individuals were analysed in the drug lists 1970-74: following the first year f purchase every full year without purchase of any antihypertensi e agent including diuretics and betablockers but with purchases of other drugs were noted If no drugs at all had been obtained for a period of time a check was made whether the patient had died migrated or been hospitalized

All persons under 60 years of age who in 1970-71 were prescribed antihype - tensive drugs by physicians in the department of internal medicine at the county hospital in Östersund were subje t to a closer examinati n The li i has no specialized hypertension unit and may be onsidered repres ntati e what regards the way most hypertensive patients in Sweden have been managed during the past years The purchase patt rn f these 46 patients 1 n and 29 women was analysed retrospe tively for the 6-year period 19 0- Knowledge of the obtained quantity of a drug and the prescribed d sage permitted calculation of the period of time that the drug sho ld last The antihypertensive drugs were missing for some time p rchases of other drugs

were taken as an indication that the patient resid d in the county all the sec Medical records were checked f r informati n on th numbe of physicians the patient had a n drug treatment prescribed the reason f r discontinuance of drugs and also blood pre sure (B P) valu s obtained in an unstandardized way n vi its as outpatients Systolic B P < 160 and diastoli B P < 95 mm Hg were consid red as good control of B P > 1 5 or > 115 mm Hg meant bad control f B P values in between indi t d acceptabl control of B P No pati nt r phys cians were int rviewed

Res lt

The xaminati n f purchas s f all antihypertensive drug made by the 916 persons re aled that every fourth individual obtained no such drug for ne y ar o more of th obs rvation period f llowing the first purchas (Figure 1) 2 f the men and 27% f th wom n were thus without treatm nt f r 20-50% f th tim of bs rvation

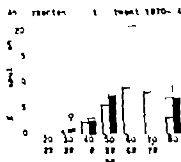


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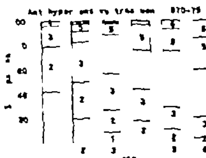


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The 46 p tient iting th int rnal d partme t had s en o rec i d prescripti ns from betwe n 2 and 20 phy i ans (m an 6 5) du ing an av rage bs rvati tim f 4 8 y ars
The umb f diff rent antihyp rt ns e drugs pres ibed f each ind vidual i combi ation r e sively d ring th bs rv ti n p iod re pres nted i Figure 2 In the f rst y ar th maj rity 60% of th p ti t were prescribed two drug nly th third y ar thi was tru f only one fou th f th patient At th end f th obs rvation period f r more drugs had b u ed by 60% f th gro p

Th antihype tensi drugs used i th p t ts are s i Figure 3 In the di al record th aus f d co t nu ng drug was ft n but far from alway identifi bl About half f th tim inadequat ff ct ad re a ti was t ted I abo t ery fifth case non-complian was id nt whil in the remaind r th us could n t b establi hed although igns f l cking cont nuity i th are f th patients were freque t

I Figure 4 th ff t f the bl od pressure tre tm t is seen in relation to th continuity f drug tre tment and th continuity of phy i ians involved One f rth (26%) f th un le ted patient had a good control f th i B P at th d f th b rv ti n period abo t two thirds (65%) were cceptably trolled and th remaini g 11% were bad control Th prop rti n f the b rv t time with t drug t eatment i periods when the amount f di pensed drug i relation to th pres ribed dose l arly indicated that no drug tre tment uld ha been going on is fo eh

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Introduction

In the long-term treatment of hypertension or any other chronic illness patients may evade treatment and/or control visits for a period of time or they may refuse cooperation completely In other patients in spite of a seemingly good compliance, the blood pressure cannot be controlled with any regimen There are a number of reasons for these therapeutic failures (3 5) some of which are quite feasible to overcome (4 6)

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were taken as an indi ation that th patient resided in the county all the time. Medical records were checked f inf rmation on th numb r f physicians the patient had seen drug treatm nt prescribed th reason for discontinuance f drugs and al bl od pre sure (B P) valu s btained in an unstandardized way n visit as outp tients Systoli B P < 160 and diastoli B P < 95 mm Hg were consid red as good control f B P > 175 or > 115 mm Hg meant bad control f B P values in between indicated ac pt ble control f B P N patient or physicians were int rviewed

Results

The examination of purchas of all antihypertensive drugs mad by the 916 p rsons revealed that ev ry fourth ndividual obtained no such drug f r one year r more f the ob rvation period following the first purchase (Figure 1) 22 f th men and 2 % f the wom n were thus without treatment for 10-50% of the time of b rvation



Fig 1 Age and sex d strib t on of 916 p rsons re iving ap ti fied antihypertens ve drugs 1970-74 One fourth of th pati ts (top f each bar) had treatme t gap f n y ar more

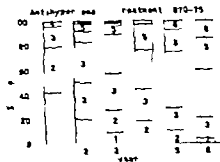


Fig 2 Numbe f antihyp rt ns drugs (nclud ng b tablockers and diureti) used b 46 pat nt Th num f drugs ach y ar i cumulated from preced ng ars

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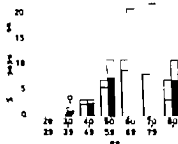


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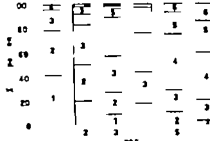


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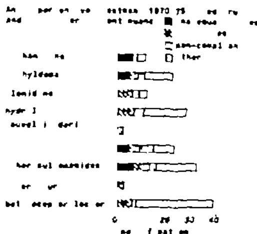


Fig 3 Antihypertensive drugs alone or in combination in 46 patients The reason for discontinuing a drug was obtained from medical records

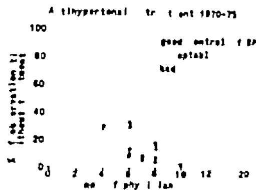


Fig 4 Effect of treatment in 46 patients in relation to continuity of treatment and physicians involved

individual along the vertical axis About half of the patients spread rather evenly between 0 and 20% Most of the remainders had less than 40% of the time untreated The effect of treatment on the B P did not seem to be affected by the degree of non-treatment in these patients Along the horizontal axis the number of physicians involved is shown Patients with good control of B P visited on an average fewer doctors than did patients with less good control If the number of prescribed antihypertensive drugs per individual was instead shown along the horizontal axis it would be seen that none of the good controls required more than four different drugs during the observation period

Two examples of antihypertensive treatment pattern will be given Patient I (Figure 5) was prescribed antihypertensive drugs by four physicians during the six years The rather sporadic purchases leaves almost half of the observation time without drug treatment Patient II (Figure 6) on the contrary, has been generously supplied with drugs from altogether 11 physicians leaving few days untreated

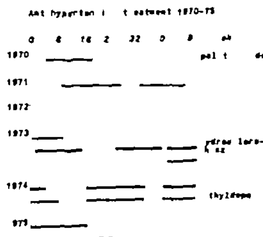


Fig 5 Purchase pattern 1970-75 in patient I Beginning of each line indicates time of dispensation length of line signifies the period of time the dispensed quantity should last

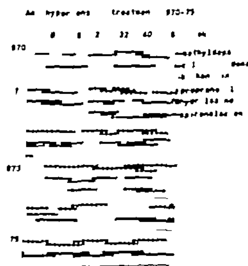


Fig 6 Pur h s pattern 19 0- in patie t II

Discussion

Some methodol gical factors must b remamb red in evaluating th finding presented here First a drop out f less than 5% in th coding of prescriptions exi ts Thus om antihyp rtensiv drugs may ha been dispensed within th unty t th patients without our knowledge Al o som drugs may hav b en obtained ut ide th county g while on vacation 8 cond th phy ician and hi patient may agre n a chang in dosage which will not be reveal d in ur data until a new prescription is filled and th new d sage recorded This increase d as in dos naturally aff cts the length f time that th di pen ed supply of drug will last Third and mo t obvious littl is known about the a tual ingestion f btained drug Howev r th se res rvations do not chang th overall imp essi that th i a la k of continuity in th treatm nt f hypert nsion in th p tients in thi t dy

In th survey f 916 p rs ns re eiv ng antihypertensive tr atment it was found that fourth of the pati nts had wid gap n th ir drug treatment Som explanat ns ar concei bl treatment t rted n to weak an indicati n; di continuity in th medi al taff f th county; non-compl an from th patient Th importanc of any f th se fa tors cannot be known without an analy is f medical cord Th se rec rd studies n the 46 p tients l arly howed that al interviews with do tors and pa ti nts are often n ssary if compl te knowledge i to b btained

An analysi of pre ripti n d ta ann t gi e a compl te pi ture f the clinical cours f a dis ase r all aspe t of ts tre tment Su h d ta may howev contribute in larifying q antitat vely the problems nvol ed in th treatment of different ndit ns sp ally chroni di eases like hypert nsi n What pat nt we hould tart n treatment with what drugs how int ns ly and fo how long may still b discus ed for many years n It i evid nt from th pre entation though that the p tients we d le t f treatm nt h uld be tak n are f i bett way than hith rto

Acknowledgement

The project p nsored by grants from th Nat onal Corporatio f Swedish Pharmaci and the County Coun il f Jämtland

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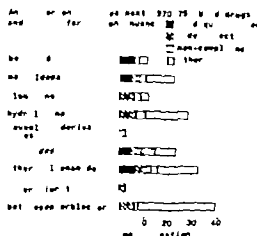


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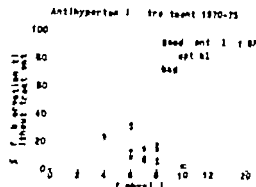


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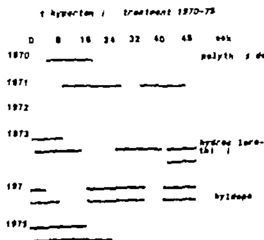


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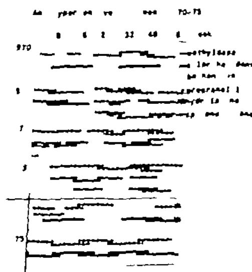


Fig 6 Pur hase patt rn 1) 5 ir patie t II

Discussi n

Some method logical factors must be rememb red in valuating the findings presented h re First a drop out of les than 5% in the coding of prescriptions exists Thus some antihypertensive drugs may hav been di pensed within the county to th s pati nts without our knowledge Al o some drugs may ha been btained out ide th county e g while n vacation S cond the phy i ian and his p tient may agree n a change in dosage which will not be re aled in ur data until a new pres ripti n is filled and the new d sag recorded This in reas or d creas in d se naturally affects th length of tim that th di p nsed supply of drugs will last Third and mo t obvious littl i known abo t the a tual ingestion f obtain d drug Howev th s re ervations do not change th *overall impression that there is a lack f continuity* in the treatm nt f hypertension in th patients in thi study

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Acknowledgement

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Islet pathology – Islet function –
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insulin

ISLET PATHOLOGY
ISLET FUNCTION
INSULIN TREATMENT

Edited by Rolf Luft

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Mölndal Sweden 1976

Preface

The great breakthrough at the turn of our century in understanding diabetes was the demonstration that acute diabetes followed pancreatectomy Thereafter the isolation and purification of insulin brought life to diabetics who without it faced death During the following three decades investigation in diabetes was primarily directed towards improving regulation of blood sugar principally by developing for clinical use a multiplicity of different insulin preparations Towards the end of this period the introduction of the oral hypoglycemic agents seemed to give new hope for normalization of blood glucose in adult-onset diabetes without the need for parenteral administration

The mid-century opened new vistas The necessity for enhanced insight into the fundamental nature of diabetes was made increasingly evident by the observation that reversing severe hyperglycemia and ketoacidosis and prolonging the life of diabetics resulted in a different clinical problem an increasing manifestation of the complications of specific diabetic vascular disease Immunology played its part with the unexpected demonstration that virtually all insulin-treated diabetics produced insulin-binding antibodies This led to the development of the new tool radioimmunoassay upon which much of the diabetes investigation during the past two decades has been dependent. For the first time we are able to measure and thereby to understand the interrelationship between the action of different hormones and glucose homeostasis

The last decade has seen an exponential increase in fundamental research and knowledge related to diabetes Among the significant contributions are the discovery of the mechanism for biosynthesis of insulin increased understanding of insulin secretion in the diabetic prediabetic and normal states which inevitably leads to deeper insight into the pathogenic mechanisms of the disease and of increasing indica

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tions of diverse causal factors in the etiology of diabetes All these and many more are the achievements of these past few years

This book is designed to give a few of those who have participated in these developments the opportunity to share with the clinician the clinical investigator and the basic scientist some of their thoughts on the past present and potential of diabetes research and therapy It has been entitled Insulin to emphasize the central role of relative or absolute insulin deficiency in the clinical syndrome – diabetes

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This book dedicated to insulin was planned during the 50th Anniversary Celebration of the Nordisk Insulin laboratorium and is published during the 50th *Anniversary of the establishment of the* Nordisk Insulin Foundation This foundation has supported scientific work in the Scandinavian countries within the fields of physiology and endocrinology

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The endocrine pancreas. Functional morphology and histopathology

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Insulin preparations and the clinical use of insulin

Jacob E. Poulsen Torsten Deckert

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General introduction

The search for the origin of diabetes has been characterized by a continuous desire to locate anatomically the basis for the observed pathological and physiological phenomena. Although the diabetic syndrome was already recognized early in the history of mankind, it was not until 1889 (von Mering and Minkowski) that its relation to the pancreas was firmly established. But only a few years later it was demonstrated that glucose homeostasis was highly dependent on the endocrine function of the islets of Langerhans (Hedon 1892, Laguesse 1893).

These discoveries led to pathological studies which provided evidence for a relationship between alterations of the islets and diabetes. A better understanding of the physiology of the islets of Langerhans was obtained from morphological and physiological studies. A and B-cells were identified by Lane (1907) and Bensley (1911). The extraction of pancreatic protein which decreases hyperglycemia in vivo (Banting and Best, 1922) initiated studies leading to the localization of the insulin secretion in the B-cells (Dunn et al. 1943 Lacy 1959) whereas the hyperglycemic hormone glucagon was located in the A-cells (Baum et al. 1962).

Despite intensive work and many historical discoveries, the etiology and the pathogenesis of diabetes mellitus remains mysterious. The origin of the failing insulin secretion in elderly diabetics is not adequately explained by the changes in their islet tissue. The cause of the progressive disappearance of the B-cells from the pancreas of young diabetics remains unknown.

It is the purpose of our contribution to review the information which has become available since the beginning of this century in relationship to the histophysiology and the histopathology of the endocrine pancreas in diabetes. For the less recent information, we shall rely heavily on several earlier excellent publications on these subjects (Kraus 1929, Lazarus and Volk, 1962, Warren et al. 1966).

Histology

1 The islet tissue

The endocrine part of the mammalian pancreas is composed of small clusters of cells the islets of Langerhans irregularly distributed in the pancreatic parenchyma The number of islets is quite variable but has been estimated at about 1 000 000 for the pancreas of most adult humans (Ogilvie 1937) The total volume of the islet tissue represents 2 to 3 % of the whole gland and the total weight amounts to 1 or 2 gr in adult humans (Mac Lean and Ogilvie 1955 Gepts 1957 1958)

The islets vary considerably in size averaging 100 to 200 μ in diameter Small islets are more numerous but the major part of the endocrine pancreas corresponds to medium-sized islets (Hellman 1959)

Each islet is supplied by one to three arterioles and drained by one to six venules (Bunnag et al 1963) The islet cells are separated from the blood stream by the largely fenestrated endothelium of the islet capillaries and by two basement membranes one closely applied to the epithelial cells the other underneath the endothelium In between normal islets show a few pericytes fibrocytes histiocytes and mast cells (Westermark 1973)

The information about the innervation of the islets is rather vague partly due to the marked species differences Both cholinergic and adrenergic fibres have been described by histochemical and electron microscopic techniques (Coupland 1958 Bencosme 1959 Cegrell 1963 Legg, 1967 Esterhuizen et al 1968 Watari 1968) Innervation does not appear an essential component of islet function although many functional studies indicate the existence of a neurogenic modulation of hormone release (Woods and Porte 1974)

2 Cellular composition of the endocrine pancreas

More than hundred years after the first description of the pancreatic islets by Paul Langerhans our knowledge of their cellular composition is still incomplete Already in 1907 Lane demonstrated the existence of the two main types of islet cells the A and the B-cells A third type the D-cell was described by Bloom in 1931 In the pancreas of normal adult humans B-cells contribute for about 75 % to the total islet cell population, whereas A and D-cells represent respectively 20 % and 5 % However many pathological conditions besides diabetes may cause significant variations in these figures

Several excellent staining techniques exist for the demonstration of the B-cells in preparations for light microscopy namely the chromium-hematoxylin and aldehyde fuchsin procedures of Gomori (1939 1941 1950) the Victoria Blue method of Ivic (1959) and the pseudo-isocyanine technique of Schiebler and Schiessler (1969) For the demonstration of A and D-cells, these methods are less satisfactory since in the Gomori procedures both A and D-cells are stained in red with phloxine To distinguish between these two types of non B-cells a thrichrome staining can be used (Bencosme 1957 Lazarus and Volk 1962) although in our experience the D-cells often fail to come out clearly

A wide variety of silver impregnation techniques has also been applied to the endocrine pancreas with the aim of demonstrating the non B-cells more distinctly The empiric nature of these silver techniques their capriciousness and the fact that they do not all impregnate the same type of cells have led to a considerable confusion in the nomenclature of the islet cells The specificity of some of the silver techniques for the demonstration of A-cells has been questioned Creutzfeldt (1953) and Gepts (1957) argued that the Gros-Schultze as well as the Holmes procedure do not only stain all non-B-cell but a variable number of B-cells as well On the

other hand, Hellerström and Hellman (1960) claimed that with their modification of the Davenport technique less A-cells are stained than with the Gomori methods. They called A_1-cells those cells that did take up silver and A_2-cells those that remained unstained. Another now widely used silver impregnation technique is the one recommended by Grimelius (1968). In contradistinction to the Hellerström-Hellman method, this technique mainly demonstrates A_2-cells but also a few A_1-cells.

The relationship of the A_1-cells to the D-cells of Bloom is still controversial. Most authors (Epple 1968, Fujita, 1964, 1968, Solcia and Sampietro, 1965, Greider et al. 1970) claim that these cells are identical, but divergent opinions have been expressed. Van Assche (1970) has pointed out that not all D-cells are stained by the Hellman-Hellerström silver technique and he has expressed the opinion that the D-cells of Bloom constitute a heterogeneous group of islet cells, some of which still await identification.

Studies with the electron microscope have failed to resolve immediately the confusion regarding the cellular composition of the pancreatic islets. A- and B-cells were easily recognized by all authors, but many among them disagreed on the number and nature of other islet cell types. Like (1967) even refused to recognize a third cell type and regarded the D-cell as a viable but altered A-cell. Greider et al. (1970) firmly opposed this view and presented the D-cell as a distinct and functionally independent cell type. Deconinck et al. (1971, 1972) in agreement with the light microscopic findings of Van Assche (1970) expressed the opinion that besides A- and B-cells, the islets contain two other cell types, which they non-committedly called type III and type IV cells.

A consensus on the existence of four ultrastructural types of islet cells was reached among a group of morphologists who met in Bologna, during the International Symposium on Gastroduodenal Physiology (Solcia et al. 1973). The four types of cells were called respectively: A, B, D (corresponding to the type III cells of Deconinck et al. 1971, 1972) and D_1 (equivalent to the type IV cells of the same authors). It was pointed out that cells equivalent to D- and D_1-cells are normally present in the gastroduodenal mucosa. Recently it was reported that cells identical to pancreatic A-cells also occur in the same mucosa, at least in some species (Unger and Orci 1975, Dobbs et al. 1975, Larsson et al., 1975). B-cells on the other hand have never been detected in the gut mucosa of mammals.

The ultrastructural identification of the four types of islet cells is mainly based on the characteristics of their secretory granules. In *B-cells* (Fig. 1) these granules are polymorphous and electron-dense, enclosing a wide membranous sac. Their overall diameter ranges between 350 and 500 mμ. The *A-cells* (Fig. 2) have granules with a slightly eccentric electron-dense core, separated from a tightly fitting, smooth membranous sac by a less dense peripheral part. The diameter of these granules ranges between 350 and 450 mμ. *D- (or type III)-cells* (Fig. 3) have large (450 to 800 mμ) granules of variable electron density, enclosed in a closely fitting smooth membrane. *D_1- (or type IV)-cells* (Fig. 4) are rarely encountered in normal islets. They contain small (150 to 350 mμ), rounded and electron dense granules, with a narrow electron-lucent space between the granule core and the limiting membrane.

3 Specific functions of the islet cells

It has been accepted since a long time that B-cells are responsible for insulin production and release. On the other hand, Hellerström and Hellman (1962), Petersson and Hellman (1963), Lundquist et al. (1970) have produced convincing evidence that among the A-cells only the A_2-cells secrete glucagon (for a complete review of their experiments, see Hellman and Täljedal, 1972). This leaves us with the problem of the function of the two

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Fig. 2
Ultrastructure of normal human A-cells × 21,000

the anterior lobe of the pituitary (Brazeau et al. 1973). It has therefore also been called somatotrophin release inhibiting factor or SRIF. Systematic studies with the aid of an antibody raised in rabbits against SRIF have revealed that this hormone is not only present in the hypothalamus and the pituitary but exists also in several peripheral tissues, including the pancreatic islets (Dubois et al. 1974, Luft et al. 1974, Arimura et al. 1975, Dubois et al. 1975, Hökfelt et al. 1975) the thyroid (Hökfelt et al. 1975) and the gastro-duodenal mucosa (Polak et al. 1975, Rufener et al. 1975). The immuno-histochemical reaction is not inhibited by oxytocin, vasopressin, neurophysin A, luteinizing-hormone releasing factor, thyrotropin-releasing factor, insulin, glucagon, gastrin or a synthetic tetrapeptide composed of an amino-sequence common to somatostatin, glucagon and secretin (Dubois, 1974). The discovery of somatostatin – (or somatostatin-like) cells in the peripheral organs has arisen considerable interest, because this hormone has been shown to inhibit many other secretory processes apart from growth hormone release, such as the secretion of insulin and glucagon (Alberti et al. 1973, Chen et al. 1974, Curry et al.,

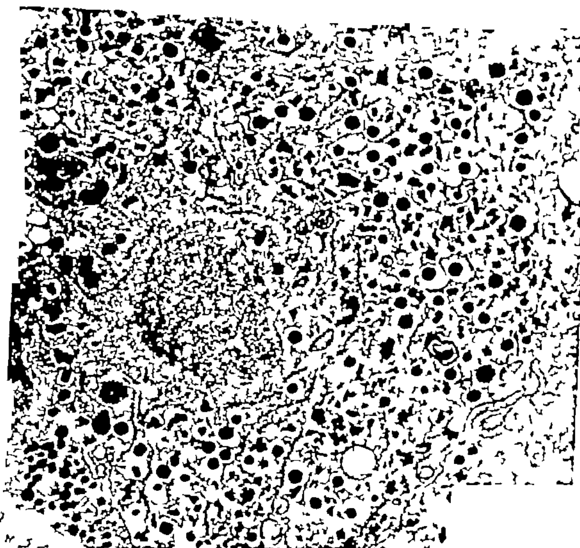


Fig 1
Ultrastructure of a normal human B-cell x 21.000

remaining cell types the D-(or type III)-cell and the D_1-(or type IV)-cell

Shortly after the identification of A_1-cells Hellman and Lernmark (1969) reported a marked in vitro inhibition of insulin release from isolated islets incubated with an extract of A_1-cell rich islets from the duck They assumed the inhibitory substance to be gastrin because Lomsky et al (1969) as well as Greider and McGuigan (1971) had detected gastrin-containing cells in the pancreatic islets with the use of fluorescent antibodies Moreover the same authors had identified the fluorescent cells as A -cells on the basis of their positive silver staining with the Hellman-Hellerström technique However recent studies from our laboratory (Lofstra et al 1974) and by other authors (Creutzfeldt et al 1971 Pearse 1975 Hökfelt et al 1975) have failed to identify gastrin cells in the islets of normal humans

A more likely candidate for secretion by the D-(or type III)-cell has been discovered recently somatostatin This hormonal tetradecapeptide isolated from the hypothalamu inhibits the release of growth hormone from



Fig. 2
Ultrastructure of normal human A-cells x 21.000

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Fig. 3
Ultrastructure of a D-(type III) cell and of a B cell ×21,000

1974 De Vane et al 1974 Efendic et al 1974 Fujimoto et al 1974 Gerich et al 1974 a b Koerker et al 1974 Mortimer et al 1974 Sakurai et al 1974) of gastrin (Arnold et al 1975) the acid secretion of the stomach (Arnold et al 1975 Barros D Sa et al 1975) as well as the release of pancreatic enzymes (Creutzfeldt et al 1975) More particularly for the pancreatic islet the [illegible] topographical relationship of the somatostatin-containing cells with B and A-cell [illegible] kes the possibility of a local regulation of the



Fig 4
Ultr tructure of A-cell and of D (type IV) ell 21,000

release of insulin and of glucagon by these cells.

Restaining experiments have revealed that the cells reacting with somatostatin-antibodies are silver-positive with the Hellman-Hellerström technique (Hökfelt et al. 1975). Therefrom it has been concluded that these cells are A -(or D- or type III)-cells. Further confirmation has been provided at the ultrastructural level by Orci et al. (1975). With the use of the unlabelled antibody technique of

Sternberger Rufener et al (1975) were able to localize SRIF in the granules of typical D-(or *type III*)-*cells*

The function of type IV islet cells (or D_1-cells) still awaits elucidation A possible candidate for secretion by this type of cell is the pancreatic polypeptide first isolated by Kimmel et al (1968 1971) as a contaminant of chicken insulin Peptides similar to the avian pancreatic polypeptide (APP) have been isolated from bovine ovine porcine and human pancreas (Langsley et al 1973 They all have 36 amino-acid residues but *differ in amino-acid sequence Only a few of* the physiological properties of this newly isolated pancreas polypeptide are known as yet stimulation of gastric secretion and of liver glycogenolysis depression of lipolysis inhibition of pentagastrin stimulated acid secretion (Lin and Chance 197? Hazelwood et al 1973) Using a serum against human pancreatic polypeptide (HPP) Larrson et al (1974 1975) have identified HPP producing cells in the pancreas of man which we have been able to confirm in our laboratory The number of HPP-cells is quite variable from one individual to the other They occur as isolated cells or in small clusters which are more frequently located in the exocrine parenchyma than at the periphery of the islets *Most islets of the human pancreas* are completely devoid of HPP-cells which may explain why these cells have escaped attention in many ultrastructural studies The possible physiological importance of this newly discovered *pancreatic polypeptide* requires further investigation.

The considerable confusion which has existed until recently about the cellular composition *of the pancreatic islets* is progressively disappearing It remains however to be elucidated whether the juxtaposition of cells with completely different but nevertheless partly physiologically related functions is of crucial *importance* and whether each of these cells participates in a well integrated islet function The latter possibility is supported by the demonstration by Orci et al (1973 e) of areas of structural specializations of cell membranes (*gap and tight junctions*) between neighbouring islet cells of the same as well as of different type These authors suggest that gap junctions may play a role in the control of the asynchronous but coordinated activity of the different types of islet cells Maturity-onset diabetes in man a bihormonal disease not only *characterized by a reduction in insulin* response but also by an increased glucagon response could represent an expression of a failure of the functional integration of A and B-cells (Unger 1970 197?)

Functional morphology of the B cells

1 Introduction

The demonstration of an impaired insulin secretion in diabetes (Yalow and Berson, 1960 a, Cerasi and Luft, 1963 1967 a, b) has focused considerable attention on the regulatory mechanisms in the function of the B-cell and on the intracellular aspects of the insulin secretory process, with the hope of localizing the primary defect in diabetes. Helped by several excellent review articles, which have been devoted to this interesting area (Steiner et al. 1972, 1974 Randle and Hales, 1974 Lacy 1975), we have tried to assemble the various morphological aspects which appear to be involved in the B-cell response to glucose

For practical purposes, a distinction has been made between the receptor unit recognizing the B-cell regulators and the effector unit, responsible for the cell response. The possible role of microtubules and microfilaments in insulin release will be discussed separately

2. The receptor unit

The development of an insulin radioimmunoassay (Yalow and Berson 1960 b) and the isolation of viable islets of Langerhans (Hellerström, 1964 Lacy and Kostianovsky 1967) have initiated numerous in vitro studies on islet function demonstrating the regulatory role of a wide variety of physiological and pharmacological agents (Malaisse 1977 a). The existence of an entero-insular axis illustrates the in vivo modulation of hormone release by such humoral substances as nutrients and gastrointestinal hormones (Fajans and Floyd 1972), whereas the association of cholinergic and adrenergic nerve endings with A B and D-cells (Esterhuizen et al. 1968 Kobayashi and Fujita, 1969) constitutes the anatomical basis for a neural control of islet function (Woods and Porte 1974) which appears to be mediated through both chemical and electrical synapses (Orci et al 1973 a).

The mechanisms through which extracellular factors influence the intracellular events of the endocrine pancreas remain however poorly defined, although the stimulus-secretion coupling of the B-cell has received considerable experimental attention In view of receptor studies in other tissues it is generally believed that hormones and neurotransmittors modulate islet functions via interactions with membrane receptors. Such hypothetical model has been experimentally documented by Ewart et al. (1975) who demonstrated a parallelism between the binding of mushroom lectins to islets and the associated insulin and glucagon release the morphological evidence for lectin binding sites on the islet cell surface was provided by scanning electron microscopy using hemocyanin-labelled concanavalin-A (Ravazzola et al. 1975). Further studies are however required on the existence and possible activation of islet membrane receptors the recently developed method for the isolation of islet membranes (Lernmark et al 1975) will certainly contribute significantly to such analysis.

The desire to determine both the nature and the localization of the receptor unit in B-cells has initiated the search for enzyme activities *in islet sections* homogenates and subcellular fractions In addition to the enzymes involved in various metabolic pathways (Matschinsky 1972 Randle and Hales 1972) islet homogenates contain both an adenylcyclase (Atkins and Matty 1971 Davis and Lazarus, 1972 Kuo et al. 1973) and a guanylcyclase (Howell et al. 1974) system, which have been located on the plasma membrane (Howell and Whitfield, 1972) (Fig. 5) The message of extracellular factors might

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1 Introduction

The demonstration of an impaired insulin secretion in diabetes (Yalow and Berson, 1960 a, Cerasi and Luft, 1963 1967 a, b) has focused considerable attention on the regulatory mechanisms in the function of the B-cell and on the intracellular aspects of the insulin secretory process with the hope of localizing the primary defect in diabetes. Helped by several excellent review articles which have been devoted to this interesting area (Steiner et al 1972 1974 Randle and Hales 1974 Lacy 1975), we have tried to assemble the various morphological aspects which appear to be involved in the B-cell response to glucose

For practical purposes, a distinction has been made between the receptor unit recognizing the B-cell regulators and the effector unit, responsible for the cell response. The possible role of microtubules and microfilaments in insulin release will be discussed separately

2. The receptor unit

The development of an insulin radioimmunoassay (Yalow and Berson 1960 b) and the isolation of viable islets of Langerhans (Hellerstrom 1964 Lacy and Kostianovsky 1967) have initiated numerous in vitro studies on islet function, demonstrating the regulatory role of wide variety of physiological and pharmacological agents (Malaisse 1972 a). The existence of an entero-insular axis illustrates the in vivo modulation of hormone release by such humoral substances as nutrients and gastrointestinal hormones (Fajans and Floyd, 1972) whereas the association of cholinergic and adrenergic nerve endings with A B and D-cells (Esterhuizen et al 1968 Kobayashi and Fujita 1969) constitutes the anatomical basis for a neural control of islet function (Woods and Porte 1974) which appears to be mediated through both chemical and electrical synapses (Orci et al 1973 a)

The mechanisms through which extracellular factors influence the intracellular events of the endocrine pancreas remain however poorly defined although the stimulus-secretion coupling of the B-cell has received considerable experimental attention. In view of receptor studies in other tissues it is generally believed that hormones and neurotransmitters modulate islet functions via interactions with membrane receptors. Such hypothetical model has been experimentally documented by Ewart et al (1975) who demonstrated a parallelism between the binding of mushroom lectins to islets and the associated insulin and glucagon release the morphological evidence for lectin binding sites on the islet cell surface was provided by scanning electron microscopy using hemocyanin-labelled concanavalin-A (Ravazzola et al. 1975). Further studies are however required on the existence and possible activation of islet membrane receptors the recently developed method for the isolation of islet membranes (Lernmark et al 1975) will certainly contribute significantly to such analysis

The desire to determine both the nature and the localization of the receptor unit in B-cells has initiated the search for enzyme activities in islet sections homogenates and subcellular fractions. In addition to the enzymes involved in various metabolic pathways (Matschinsky 1972 Randle and Hales 1972) islet homogenates contain both an adenylcyclase (Atkins and Matty 1971 Davis and Lazarus, 1972 Kuo et al 1973) and a guanylcyclase (Howell et al. 1974) system, which have been located on the plasma membrane (Howell and Whitfield 1972) (Fig. 5) The message of extracellular factors might



Fig 5
In B-cells of rat islets of Langerhans the adenylcyclase activity has been visualized by lead precipitation of PNP which was previously formed during an incubation with 0.5 mM AMP PNP and 10 mM fluoride The precipitate was found exclusively and almost uniformly at the outer surface of the plasma membrane x 15 000
(from Howell & Whitfield 1972 courtesy J Histochem and Cytochem)

thus be transmitted through the intracellular messengers cyclic AMP or cyclic GMP which modulate insulin synthesis and/or release via an as yet undefined mechanism It has been postulated that glucose interacts also with a membrane receptor related to the adenylcyclase system (Cerasi and Luft 1970 a Matschinsky and Ellerman 1973) and that a defect in this signal transmission might represent the central defect in diabetes (Cerasi and Luft *1970 b) Although glucose* does indeed stimulate cyclic AMP formation in islets of Langerhans (Charles et al 1973 Grill and Cerasi 1974) the effects of glucose upon islet function can not be explained and obtained by cyclic AMP alone (Malaisse 1973 Hellman et al 1974) The failure to demonstrate a stimulated release by non metabolized sugars *has furthermore been used* in favor for the substrate site hypothesis in which glucose metabolism is thought to generate besides ATP a signal that triggers insulin release (Hellman 1970 Ashcroft et al 1972) The recent demonstration that the greater insulinotropic effect of α-D-glucose as compared to the β-form (Niki et al 1974 Grodsky et al 1974) is not associated with facilitated glucose transport and metabolism (Täljedal 1975) but is accompanied with increased cyclic AMP formation *(Grill and Cerasi 1975)* stresses however the view that at least part of the glucose effect is carried out throuth cyclic AMP formation

The introduction of the *freeze etching* technique in islet research (Orci et al 1973 b) has created a new tool for the study of membrane phenomena This procedure succeeds indeed in the splitting of plasma membranes exposing their inner hydrophobic matrix in which the frequency of the protein containing particles appears to reflect the metabolic activity of the membrane (Branton 1971) In an elegant work Orci and his collaborators have illustrated the possible relationship between islet functions on one hand and the number and distribution of the membrane associated particles on the other hand It thus appeared that in experimental (Orci et al 197? a) as well as in spontaneous (Orci et al 1974 a) diabetes *these particles are clustered instead* of distributed in a random pattern normal cell membranes contain also aggregated particles which can be arranged as arrays at the site of gap junctions or as ridges characterizing the tight junctions (Orci et al 1974 a) (Fig 6) The demonstration of gap junctions between the various islet cells did furthermore *furnish an ultrastructural basis for a possi*ble coordinated activity of the insulin and glucagon secreting cells (Orci et al 1973 d) It is indeed conceivable that inorganic ions and small molecules which can diffuse through these intercellular channels are responsible for simultanous cell (in)activation resulting in a syncitial response which i comparable to the coordinated contraction in muscle fibers The possible role of membr ne associated particles in the receptor unit of the beta cells *requires however further inve tig*tion but the observation that the increa ed development of tight junction after prote ly

tic enzyme treatment is associated with a facilitated glucose induced insulin release, might point into this direction (Orci et al. 1973 e).

3 The effector unit

General description

Both A and B-cells are equipped with a secretory device capable of producing a fast as well as a sustained hormone release (Grodsky et al 1967 Iversen 1971). The synthetic machinery of the beta cell can also be quickly turned on (Steiner et al 1972), whereas the time course of glucagon synthesis is still under study (Hellerstrom et al. 1974). Such immediate cell responses do not only require a fast transmission of the stimulatory signal, but also the existence of synthetic and secretory effector units in which the various participating organelles can be rapidly activated. Sustained hormone secretion will on the other hand depend on a sufficient intracellular transport of secretory material t the plasma membrane So far the intracellular processing of glucagon has not been very much explored, so that the present review will be mostly dealing with glucose stimulated B-cells. The subsequent events in insulin producing cells are however not basically different from those in other secretory cells, a similarity which has contributed largely to our current knowledge on the B-cell function. Inspired by the elegant work of Jameson and Palade (1967 a, b), several investigators have indeed demonstrated a similar intracellular processing in the B-cells (Howell et al 1969 a Howell 1972 Orci et al 1973 f) of which the morphological aspect will be briefly reviewed

P otein synth sis

In analogy to other secretory cells (Palade 1975) the secretory product of the B-cell are also formed in the tubules of the rough endoplasmic reticulum and condensed into a visible ore within the Golgi cisternae (Howell et al 1969 a Orci et al. 1973 f). These proteins correspond to proinsulin a precursor of insulin, whose synthesis can be induced by glucose (Steiner et al 1967) Glucose exerts a preferential stimulation of the translation of proinsulin messenger RNA during short term incubations (Permutt and Kipnis 1972 a). A long-term exposure to glucose increases also the transcription of new proinsulin messenger RNA (Permutt and Kipnis 1972 b) which might be associated with increased amounts of rough endoplasmic reticulum, as observed in cultured islets (Andersson et al. 1974)

The synthesis of non insulin proteins proceeds also in the absence of glucose and is much less enhanced by glucose than proinsulin formation (Permutt and Kipnis 197? a Pipeleers et al. 1973 a). Gel electrophoresis of the newly synthetized islet proteins indicates the existence of two major bands comigrating with purified brain tubulin and muscle actin (Fig 7).

Hormone transport

F om endoplasmic eticul m to Golgi-com pl x Similar to exocrine pancreatic cells both the A and B-cells channel their newly synthetized secretory proteins from the rough endoplasmic reticulum to the Golgi ap-

F g 6

F eeze-etch studies of islet ell membranes hav demonstrated different patterns in the distribution of membrane-associated particles

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(a + b from Orci et al 1974 a courtesy Diabetologia – + d from Orci et al 1974 b) ourtesy Ex erpta Medica

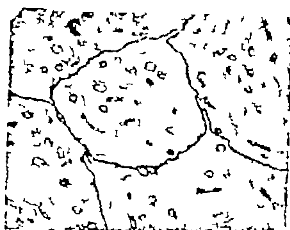


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Protein synthesis

In analogy to other secretory cells (Palade 1975) the secretory products of the B-cell are also formed in the tubules of the rough endoplasmic reticulum and condensed into a visible core within the Golgi cisternae (Howell et al. 1969 a Orci et al 1973 f) These proteins correspond to proinsulin, a precursor of insulin, whose synthesis can be induced by glucose (Steiner et al. 1967) Glucose exerts a preferential stimulation of the translation *of proinsulin messenger RNA during* short term incubations (Permutt and Kipnis, 197? a). A long-term exposure to glucose increases also the transcription of new proinsulin messenger RNA (Permutt and Kipnis 1972 b), which might be associated with increased amounts of rough endoplasmic reticulum, as observed in cultured islets (Andersson et al. 1974).

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Fig 6

Freeze-etch studies of islet cell membranes have demonstrated different patterns in the distribution of membrane-associated particles

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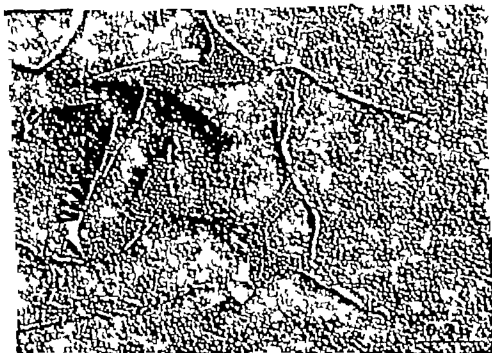
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(a + b from Orci et al 1974 a courtesy Diabetologia – c + d from Orci et al., 1974 b) courtesy Excerpta Medica





Fig. 6




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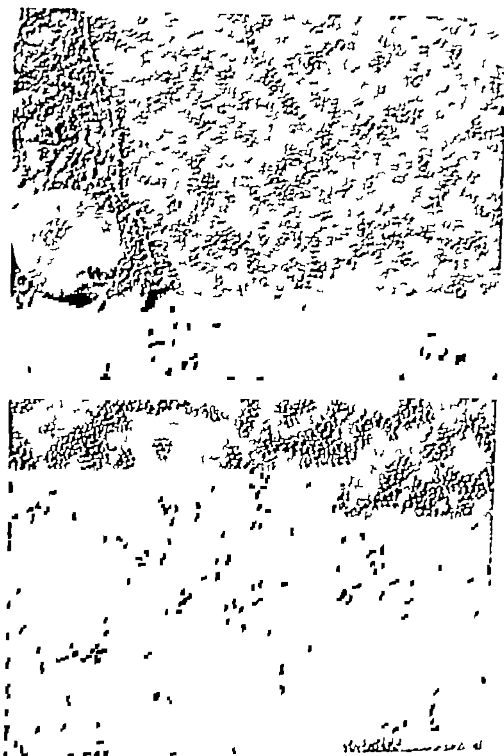
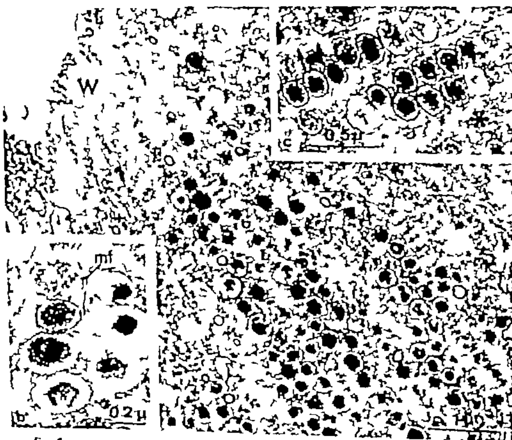


Fig. 6

Columns of three to four beta granules have indeed been described at the locus of an emlocytotic event (Lacy 1975), whereas an alignement of secretory granules has been discerned in various islet cell preparations (Gomez-Acebo and Garcia Hermida, 1974 Orci et al., 1973 f Pictet and Rutter 1972) (Fig. 8) Secretory studies suggest furthermore a non homogenous accumulation of the insulin granules which are released in a biphasic pattern upon a glucose-stimulus (Grodsky et al. 1967 Sando and Grodsky 1973) A model was therefore prepared in which the secretory vesicles are divided over two cell compartments (Grodsky et al. 1970) and in which the transport of the secretory products to the smaller compartment would represent a microtubule-dependent step (Malaisse et al. 1975) The biphasic response of the effector unit can however also be inter



F g 8
( ) Electron micrographs of B-cell grown i monolayer culture for 2.5 day Th ecretory granules appear linearly arranged nd oriented by cytoplasmic microtubules (arrows) 19 000
(b) A microfilament (mf) betw en two row of ecretory granules x 50.000
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paratus (Howell et al 1969 a and 1974) This energy requiring step (Howell 1972) has been described to occur along smooth surfaced endoplasmic reticulum budding off into microvesicles which convey the secretory products to the Golgi-complex (Orci et al 1973 f) in other tissues it has been documented however that this transport process might also occur within a continuous transition of the endoplasmic reticulum into the Golgi membranes (Claude 1970 Morré et al 1971) After 10 to 30 minutes the newly formed proinsulin reaches the Golgi tubules which have been implicated in the formation of new secretory granules (Munger 1958) Steiner demonstrated that proinsulin was converted to insulin before mature granules were formed (Steiner et al 1969) which resulted in further experimental evidence for the concept that proinsulin conversion is indeed initiated by splitting enzymes enclosed in the inner Golgi cisternae (Kemmler and Steiner 1970) The conversion of proinsulin into insulin will thus be highly dependent on the hormone transport to the Golgi-complex the observation of normal conversion rates in islets incubated in the absence of calcium, indicate therefore the existence of a normal hormone transfer in the absence of extracellular calcium and in conditions of blocked insulin release (Pipeleers et al 1973 b)

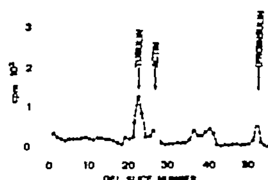


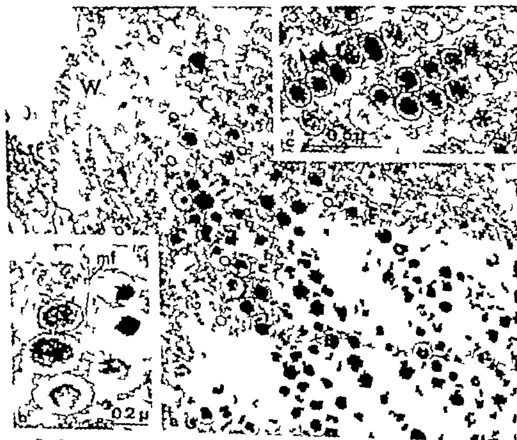
Fig 7
Gel electrophoresis of sixty islets incubated for 180 minutes in the presence of 3H-4,5-L-leucine (50 µCi/500 µl – 40 Ci/mM) and in the absence of glucose The slab gels containing 10 % acrylamide and 0.2 % sodium dodecyl sulphate are run in 0 1 % sodium dodecyl sulphate Tris glycine buffer (pH = 8.3) The pattern of the newly synthetized proteins is obtained by measuring the radioactivity of the gel slices Purified brain tubulin muscle actin and bovine insulin are used as marker molecules
(Pipeleers Pipeleers Marchal and Kipnis unpublished observations)

From Golgi-complex to plasma membrane
Secretory vesicles emerge from the Golgi apparatus where the newly synthetized products are packaged into the granule core and surrounded by Golgi membranes Several authors have described these newly formed vesicles as pale and lucent granules but such characteristic feature could not be confirmed by radioautography or by proinsulin assay (Howell 1971) It is nevertheless believed that the newly formed granules undergo a maturing process over the next 45 to 150 minutes (Howell et al 1969 a Orci et al 1973 f) during which the conversion process will continue within the secretory vesicle (Kemmler et al 1973) No preferential release of newly formed (pro)insulin molecules has been detected in rat isolated islets (Sando and Steiner 1972 Sando and Grodsky 1973) which is consistent with the concept that all secretory proteins are transported and released via a similar mechanism

Little information is so far available on the fate of the secretory vesicles after they left the Golgi-complex and before they fuse with the plasma membrane It is generally conceived that newly formed granules are pinched off from the Golgi tubules and mix with a pool of pre-existing storage granules out of which they would migrate to the plasma membrane in a random fashion (Sando and Steiner 1972)

Morphologic observations are however rather in support of a non random organization and release of the secretory granule

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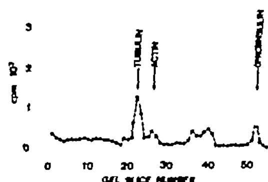


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Gel electrophoresis of sixty islets incubated for 180 minutes in the presence of H-4,5-L-leucine (50 μCi/500 μl – 40 Ci/mM) and in the absence of glucose The slab gels containing 10 % acrylamide and 0.2 % sodium dodecyl sulphate are run in 0.1 % sodium dodecyl sulphate-Tris glycine buffer (pH = 8.3) The pattern of the newly synthetized proteins is obtained by measuring the radioactivity of the gel slices Purified brain tubulin muscle actin and bovine insulin are used a marker molecules
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in vitro (Hellman, 1975) which raises the attractive possibility that granule-associated calcium (Herman et al 1973) regulates the stability and the discharge of the secretory granule. Preliminary results have also indicated that glucose can activate the solubilization of insulin granules in vitro in the presence of islet membranes (Davis and Lazarus, 1975). The development of in vitro systems for secretion thus represents a new approach to the study of the intracellular aspects of the secretory mechanism, but the inability to prepare highly purified subcellular fractions in sufficient quantities might at this moment hamper the analysis of the insulin releasing process.

4 Intracellular motility and islet function

Introduction

Since B-cell granules were released in tandem at specific loci on the cell membrane, Lacy suggested the existence of an intracellular migration of secretory products to these loci (Lacy 1961) A few years later Lacy et al. demonstrated the presence of microtubules in B-cells, which were implicated in the mechanism of insulin release based on the inhibitory effect of colchicine upon the hormone release in analogy to the participation of microtubules in chromosome movement, it was therefore postulated that a microtubular-microfilamentous network constitutes the skeleton for a vectorial transport of secretory granules to the plasma membrane (Lacy et al 1968) An association of intracellular motility and the secretory process, had also been suggested by the introduction of the term "stimulus-secretion coupling" as the counterpart for excitation-contraction coupling in non myogenic cells (Douglas, 1968). The similarity between calcium movements during secretory and contractile processes had indeed favoured the idea of the participation of contractile elements in the secretory mechanism. The existence of actin and myosin in secretory cells (Abramowitz et al. 1977 Blitz and Fine 1974) further elaborates this hypothesis. The mechanism whereby microtubules and microfilaments participate in the secretory process is not yet understood and the evidence for their role in insulin release is still indirect.

Microtubules

The use of glutaraldehyde as a tissue fixative (Sabatini et al 1963) has generalized the presence of microtubules in most eukaryotic cells indicating the existence of cytoplasmic microtubules in addition to the mitotic microtubules (Porter 1966 Behnke and Forer 1967). The observation that colchicine exerts its antimitotic effect by disrupting microtubules and inhibiting their formation (Inoué, 1964) was soon applied to non-dividing cells in order to determine the role of cytoplasmic microtubules. It thus appeared that colchicine inhibited also such diverse processes as secretion (Lacy et al 1968), intracellular transport (Malawista, 1965 Dahlstrom 1968) receptor motility (Oliver et al. 1974) and cellular mobility (Bhisey and Freed, 1971) The lack of direct evidence for an active role of microtubules and the detection of secondary effects caused by microtubule disruptive agents (Wilson et al 1970 Mizel and Wilson, 1972) should however lead to some caution in the interpretation of these results.

The postulated role of microtubules in the insulin secretory mechanism is also merely based on the inhibitory effects of colchicine and vinblastine upon the hormone release (Lacy et al. 1968 Malaisse *et al. 1971) The* initial phase of insulin release remained however intact after exposure to microtubule disruptive agents, which led Lacy to postulate that this first phase is composed of insulin granules which are already associated with the microtubular-microfilamentous system in contrast to the other "free" granules (Lacy et al 1972). This dissociation between the early and late components of hormone release might also be attributed to the fact that the

preted as a reflection of a possible biphasic pattern in the *receptor unit activity* rather than as a consequence of the participation of two distinct storage compartments (Cerasi et al 1974)

Emiocytosis

The emiocytotic process has been originally described by Palade in the pancreatic acinar cells which appeared to discharge their secretory proteins following the fusion of the zymogen granule membrane with the plasma membrane (Palade 1958) Shortly thereafter Lacy extended this phenomenon to the beta cells in which the frequent occurrence of emiocytosis in tandem was emphasized (Lacy 1961) *Electron microscopic studies in* other tissues soon introduced the concept that all secretory products encased in granule membranes were released by emiocytosis or exocytosis independently of the cell type or the secretory stimulus (Douglas 1968) It also appeared that calcium was a universal prerequisite for this release mechanism (Rubin 1970) *which initiated an intensive search* for the site of action of this divalent cation

As has been postulated in other secretory cells (Douglas 1968) the calcium dependency of insulin release is thought to originate from the need of the cytosolic calcium concentration to increase in order to obtain a stimulated *emiocytosis* (Malaisse and Malaisse Lagae 1970 Lacy 1970) In analogy to the excitation-contraction coupling in muscle cells the rise of cytosolic calcium above treshold values could activate a contractile system responsible for the extrusion of the secretory products (Malaisse 1973) Although a causal relationship has not been demonstrated between measured cytoplasmic calcium concentrations and insulin release the insulinotropic effect of various agents has been explained on the basis of alterations in calcium influx or efflux and of translocations in intracellular calcium (Malaisse 1973) Intracellular calcium is concentrated in the islet mitochondria and secretory granules (Herman et al 1973 Howell et al 1975) but rapid changes in calcium accumulation were only observed in the mitochondria suggesting their possible role in the acute regulation of *intracellular calcium levels* (Howell et al 1975) The search for the possible action site of this divalent cation has been conducted in various secretory systems and into various directions as well A role of calcium has been located in the receptor unit in view of its effects on cyclic AMP or cyclic GMP formation (Robison et al 1971 Schultz et al 1973) whereas both the packaging (Selinger 1975) and the discharge (Douglas 1974) of secretory granules have been proposed as possible target functions for calcium Assuming the existence of a steep calcium gradient *following a stimulated calcium influx* it is conceivable that the high calcium concentration in the vicinity of the cell membrane causes a decrease in the electrostatic energy barrier to the interaction between the plasma membrane and the peripheral secretory vesicles (Matthews 1970) The peripheral cell web (Orci et al 1972 b) consisting probably of *actin microfilaments* (Gabbiani et al 1974) should certainly be considered as a potential calcium sensitive organelle which could participate in the emiocytic event by pulling the secretory granules to the plasma membrane (Lacy 1975) *by interacting with myosine* like components of the granule membrane and thus opening the way to the plasma membrane (Berl et al 1973) or by simply representing a removable barrier to the plasma membrane (Orci et al 1972 b)

In order to determine the conditions causing the rupture and subsequent discharge of the secretory vesicles procedures have been developed to study secretory granules in vitro (Coore et al 1969 Howell et al 1969 b) Islet secretory granules did not solubilize upon exposure to various insulinotropic factors indicating that the intracellular discharge of secretory proteins is not likely mode of insulin secretion in vivo (Howell et al 1969 c) Calcium appears to contribute significantly to the stability of secretory granules

in vitro (Hellman 1975) which raises the attractive possibility that granule-associated calcium (Herman et al. 1973) regulates the stability and the discharge of the secretory granule. Preliminary results have also indicated that glucose can activate the solubilization of insulin granules in vitro in the presence of islet membranes (Davis and Lazarus 1975) The development of in vitro systems for secretion thus represents a new approach to the study of the intracellular aspects of the secretory mechanism but the inability to prepare highly purified subcellular fractions in sufficient quantities might at this moment hamper the analysis of the insulin releasing process.

4 Intracellular motility and islet function

Introduction

Since B-cell granules were released in tandem at specific loci on the cell membrane Lacy suggested the existence of an intracellular migration of secretory products to these loci (Lacy 1961). A few years later Lacy et al demonstrated the presence of microtubules in B-cells, which were implicated in the mechanism of insulin release, based on the inhibitory effect of colchicine upon the hormone release in analogy to the participation of microtubules in chromosome movement it was therefore postulated that a microtubular-microfilamentous network constitutes the skeleton for vectorial transport of secretory granules to the plasma membrane (Lacy et al 1968). An association of intracellular motility and the secretory process, had also been suggested by the introduction of the term "stimulus-secretion coupling" as the counterpart for excitation-contraction coupling in non myogenic cells (Douglas, 1968) The similarity between calcium movements during secretory and contractile processes had indeed favoured the idea of the participation of contractile element in the secretory mechanism. The existence of actin and myosin in secretory cells (Abramowitz et al 1972, Blitz and Fine 1974) further elaborates this hypothesis. The mechanism whereby microtubules and microfilaments participate in the secretory process is not yet understood and the evidence for their role in insulin release is still indirect.

Microtubules

The use of glutaraldehyde as a tissue fixative (Sabatini et al. 1963) has generalized the presence of microtubules in most eukaryotic cells, indicating the existence of cytoplasmic microtubules in addition to the mitotic microtubules (Porter 1966 Behnke and Forer 1967). The observation that colchicine exerts its antimitotic effect by disrupting microtubules and inhibiting their formation (Inoué 1964) was soon applied to non-dividing cells in order to determine the role of cytoplasmic microtubules It thus appeared that colchicine inhibited also such diverse processes as secretion (Lacy et al 1968) intracellular transport (Malawista, 1965 Dahlström, 1968) receptor motility (Oliver et al 1974) and cellular mobility (Bhisey and Freed 1971) The lack of direct evidence for an active role of microtubules, and the detection of secondary effects caused by microtubule disruptive agents (Wilson et al. 1970 Mizel and Wilson, 1972), should however lead to some caution in the interpretation of these results.

The postulated role of microtubules in the insulin secretory mechanism is also merely based on the inhibitory effects of colchicine and vinblastine upon the hormone release (Lacy et al 1968 Malaisse et al. 1971). The initial phase of insulin release remained however intact after exposure to microtubule disruptive agents which led Lacy to postulate that this first phase is composed of insulin granules which are already associated with the microtubular-microfilamentous system in contrast to the other "free" granules (Lacy et al 1972) This dissociation between the early and late components of hormone release might also be attributed to the fact that the

first phase granules are already located underneath the plasma membrane whereas the second phase granules are still to be transferred to the periphery via the microtubular system (Malaisse et al 1975) It is thus conceivable that the decrease in microtubule content observed in islets from spiny mice (Malaisse Lagae et al 1975) or from fasting rats (Pipeleers et al 1976) is responsible for an impaired hormone transport and consequently for the associated impairment in the insulin secretory response

In order to analyze the eventual role of microtubules in islets biochemical studies have been focused on the islet microtubular system (Montague et al 1975 Pipeleers et al 1975) In analogy to brain microtubules islet microtubules represent also polymers of tubulin which exhibits in its depolymerized form a high affinity for colchicine The rapidity of microtubule assembly and disassembly (Inoué et al 1974 Cande et al 1974) forms however a critical obstacle for the isolation and in vitro study of cellular microtubules as well as for the measurement of the tubulin polymerization in vivo A recently developed technique for the stabilization and quantitation of both polymerized and depolymerized tubulin in islets (Pipeleers et al 1976) has been used to determine whether a dynamic equilibrium between both tubulin forms represents a regulatory site for microtubule dependent functions as was originally postulated by Inoué (1964) Using this method it has been shown that glucose induces a tubulin polymerization in islets independent of extracellular calcium. Islets from fasting rats did not respond to glucose by a tubulin polymerization which paralleled a suppressed insulin secretory response the chronic administration of glucose prevented however the decrease in polymerized tubulin levels as well as in the glucose induced insulin release whereas the addition of theophylline to the fasting islets restored both the glucose-induced tubulin polymerization and insulin release (Fig 9) It was demonstrated subsequently that tubulin synthesis in islet is

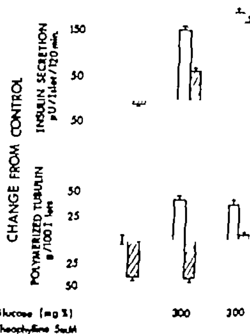


Fig 9
The islet microtubule levels were found to vary with changes in total tubulin and/or in the degree of tubulin polymerization An increase (decrease) in polymerized tubulin was accompanied with an increased (decreased) insulin secretory capability of islets isolated from fed (open columns) and fasted (hatched columns) rats and incubated for 120 minutes with or without glucose or theophylline (From Pipeleers et al 1976 courtesy Science — copyright 1976 by the American Association for the Advancement of Science)

impaired during fasting but that cyclic AMP can correct this defect (Pipeleers unpublished observations) The microtubular apparatus of rat islets might thus represent a dynamic system through which glucose and cyclic AMP can alter the insulin secretory response Further studies are now required to determine how increased level of polymerized tubulin can initiate an increased secretory activity Although a facilitated hormone transport is a likely possibility it should be kept in mind that microtubule dependent membrane functions might also regulate certain islet functions (Wunderlich et al 1973 Oliver et al 1974)

Microfilaments

The analogy between the excitation-contraction coupling in muscle cells and the stimulus-secretion coupling in secretory cells, has stimulated efforts to demonstrate the existence of a contractile apparatus in secretory tissues. Various biochemical techniques were therefore used to detect the presence of actin, myosin and their associated proteins whereas electron microscopic studies focused on the eventual occurrence of 50-80 Å microfilaments similar to the actin filaments in muscle.

In islets of Langerhans, the presence of actin has been suggested by the positive immunofluorescent reaction obtained with actin antiserum (Gabbiani et al. 1974) and by the existence of an islet protein, co-migrating with muscle actin on SDS-gel electrophoresis (Fig. 7). Recent reports have also indicated the presence of myosin in islets, which cross-reacts with antibody to smooth muscle myosin (Ostlund and Kipnis 1975). The presence of contractile structures in B-cells of the rat pancreas was also suggested by ultrastructural studies revealing a cortical band of microfilaments each measuring 50 to 70 Å in diameter (Orci et al 1972 b) The endocrine pancreas is thus likely to be equipped with an actomyosin-like apparatus, just as is the case in many other non-muscle cells (Pollard 1973 Hepler and Palevitz, 1974).

The evidence for the participation of the microfilamentous network in the insulin secretory process is however indirect, and its action mechanism still very hypothetical The implication of microfilaments in secretory functions is indeed mainly based on experiments carried out with cytochalasin, a fungal metabolite which has been described to disrupt the microfilamentous cell web beneath the plasma membrane (Schroeder 1969-Wessels et al. 1971). Whereas this treatment results in a decreased release of growth hormone (Schöfield 1971) of catecholamines (Poisner 1972) and of thyroid hormone (Williams and Wolff 1971) an increased secretion rate is observed in pancreatic B-cells (Orci et al. 1972) in leucocytes (Zurier et al. 1973) and in platelets (Haslam et al 1975). Based on the enhancing effect of cytochalasin B on glucose-induced insulin release which was associated with a "disruption" of the microfilamentous network Orci et al postulated that this network forms a barrier between the secretory granules and the plasma membrane which upon removal accelerates the access of hormone to the cell periphery Although it is generally accepted that cytochalasin B inhibits a wide variety of cellular movements (Carter 1967) it is not at all certain that this effect is due to a disruption of actin-like structures (Goldman 1972 Forer et al 1972) So far cytochalasins have been found to impair the microfilamentous system as well as membrane functions and sugar transport (Copeland 1974) In B-cells both a decreased glucose transport (Mc Daniel et al. 1974) and an altered microfilamentous network (Orci et al. 1972) characterize the exposure to cytochalasin B The corresponding potentiation of glucose-induced insulin release is however observed independently of an impaired glucose transport which occurs only after a preincubation period with the fungal metabolite (Orci et al. 1972 Lacy et al. 1973 Mc Daniel et al. 1974). The morphological alterations in the microfilamentous network in cells of established lines appear also independent of hexose uptake develop immediately after addition of the mold alkaloid and are rapidly reversible (Miranda et al., 1974 a) in complete analogy to the effects of cytochalasin on glucose-induced insulin release (Orci et al. 1972, Lacy et al. 1973 Van Obberghen et al 1973). Miranda s detailed morphologic studies on cells of established lines suggest furthermore that through a membrane interaction, the cytochalasins induce a sustained contraction of the microfilaments such persisting contraction of the microfilaments such persisting contracture would result in the morphological appearance of tightly packed filamentous masses, which could be interpreted as disrupted microfilaments. It is thus appealing

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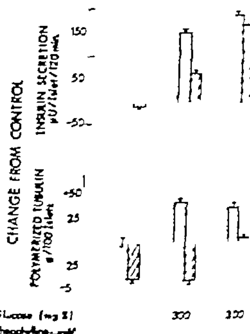


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to postulate that such hypercontractile state facilitates the glucose-induced insulin release by the B-cell All available evidence points to the active role of an actomyosin system in secretory processes but further direct evidence is required to accept this attractive hypothesis

Histopathology

1 Introduction

The pathology of the islet tissue has been studied mainly for its possible bearing on the pathogenesis of diabetes mellitus. Much of our present knowledge in this area has been derived from the pioneering work of distinguished pathologists at the beginning of this century. Opie (1901), Weichselbaum (1910), Heiberg (1911) were among the first to describe the classical lesions of the insular tissue in diabetes, namely the fibrosis, hyalinosis, hydropic degeneration, atrophy and the inflammatory infiltration. The assumption that these changes, concomitant to a decrease in the number of islet cells, would afford an adequate explanation for the disease, gained almost unanimous support at first, but was dismissed later on when it became clear that the described islet lesions were neither constant, nor specific. For a long time the attention of the pathologists was then diverted to other organs whose possible relationship with diabetes was examined; these studies lead to the concept that many other hormones of non pancreatic origin, such as growth-hormone and glucocorticoids, might play an important role in the maintenance of a normal carbohydrate metabolism. A new pathogenic explanation was gradually installed, which attributed diabetes to a progressive exhaustion of the B-cells resulting from a prolonged state of hyperfunction in response to diabetogenic extra-pancreatic factors ("Gegenregula-

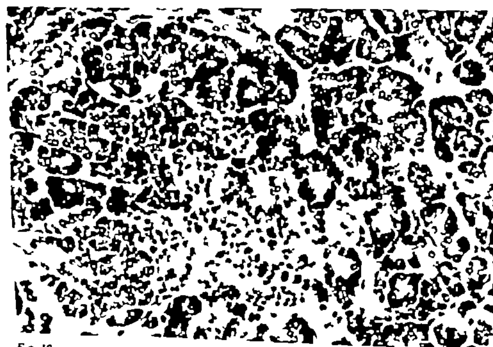


Fig. 10
Atrophic islet with lymphocytic infiltration in a recent-onset juvenile diabetic. Chromium hematoxylin-phloxin. x 450

tionsdiabetes) However significant changes in the pituitary or adrenal glands were never demonstrated in diabetics Neither could other factors capable of eliciting a relative insulin deficiency such as insulin-antibodies excessive insulin destruction or anomalies of supposed carrier proteins of insulin be decorated with any decisive importance

However several factors helped to refocus the attention of the investigators on the endocrine pancreas Histological as well as biological observations were reported suggesting that a functional anomaly of the B-cells plays an important part in the pathogenesis of human diabetes Lazarus and Volk (1962) were the first to point out that in elderly diabetics the B-cells often fail to develop signs of hyperactivity despite the hyperglycemia to which they were submitted for a prolonged time We have been able to confirm their observation repeatedly in our own material It was also demonstrated that in response to the same glucose stimulation the B-cells of diabetics react more slowly and secrete less insulin than those of non-diabetics (Cerasi and Luft 1967 a and b) This functional deficiency exists not only in overt diabetics but also in patients with genetic prediabetes and even in about 20 % of healthy subjects According to Cerasi and Luft the secretory deficiency of the B-cells is the inherited factor responsible for diabetes In the majority of cases the disease would become overt under the influence of added diabetogenic factors with which the genetically deficient B-cells can not cope

This important finding together with the introduction of many new and refined techniques helped to reawake the interest in the structural and biochemical organization of the pancreatic islets Unfortunately for ethical and technical reasons it remains very difficult to apply these new techniques to the study of the human pancreas Therefore not so much has been added in recent years to what the older authors have taught us about the pathology of the pancreas in diabetes However the significance of certain lesions which had been underestimated or misunderstood by the first students of the diabetic pancreas became to be reevaluated in the light of a better understanding of the physiopathology of diabetes and of new facts in relation to the epidemiology of the disease

2 Qualitative changes of the islet tissue in diabetics

These changes may affect the whole islets the islet cells and the islet stroma

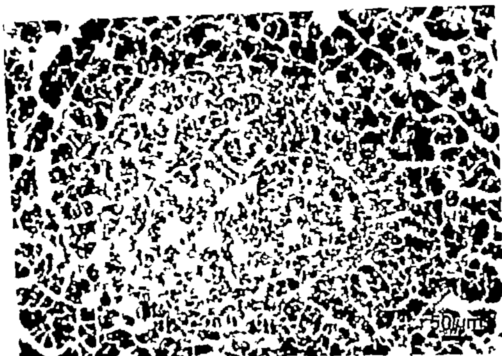
Atrophy and hypertrophy of the islets

Atrophy represents the prevailing change in the islets of early-onset diabetics (Gepts 1965) The atrophic islets are composed of thin cords of cells arranged in a fibrous stroma or separated by capillaries (Fig. 10). The islet cells are small and contain a small nucleus with dense chromatin the cytoplasm is also reduced may stain red with phloxine (A cells?) but does not show any secretory material These islets often exhibit irregular outlines and a continuity between the cords of insular cells and the surrounding acinar tissue is frequently observed.

Hypertrophy of the islets has been reported by Ogilvie (1933) as a feature of the pancreas in obesity This observation is of interest, because a pronounced degree of hyperinsulinism often exists in such patients Islets exceeding 300μ in diameter are relatively frequent also in recent-onset juvenile diabetics (MacLean and Ogilvie 1959 Gepts 1965) (Fig 11) These islets are usually composed of a majority of hypertrophic degranulated B cells but A cells are present as well We believe that this islet hypertrophy compensates for the atrophy of the majority of islets in juvenile diabetics

Changes of the B cells

Degranulation In young diabetics with a disease of short clinical duration the small number of surviving B cells are almost com



F ig 11
Hypertrophic islet with peripheral lymphocytic infiltration in a recent-onset juvenile diabetic Chromium hematoxylin-phloxine

pletely degranulated (Gepts, 1965) at least when viewed with the classical granule stains for light microscopy Application of the fluorescent antibody technique proves that these cells still contain a small amount of insulin and confirm their identification as B cell I older diabetics, degranulation of the B cell is much les marked and may even be lacking completely (Lazarus and Volk, 1962, Gepts, 1957) In the case of acute juvenile diabetics, the association of marked degranulation with nuclear hypertrophy and increased amount of cytoplasmic ribonucleic acid clearly indicates a tremendous secretory hyperactivity I contrast, the small relatively well granulated B cells of elderly diabetics seem t support the concept that maturity-onset diabetes results from failure of the B cells to react adequately to the stimulus of hyperglycemia.

Nuclear change Hypertrophy of the nuclei in hyperactive B cells of recent-onset juvenile diabetics has already been mentioned. In the same group of diabetics, irregularity hyper chromatism and pycnosis of B cell nuclei are also encountered (Gepts, 1965 Warren et al., 1966) (Fig. 1). Despite their intense hyperac tivity the B cells of acute juvenile diabetics rarely show mitoses whereas in non diabe tics and even in elderly diabetics mitoses may appear in relatively large numbers in certain pathological conditions (LeCompte and Merriam, 196_, Potvliege et al. 1963). These observations probably correspond to a de rangement of nucleic acid metabolism and an impairment of the regenerative capacity of the B cells in juvenile diabetics.

Hydropic changes Hydropic changes of the B cells are characterized by swelling, a com-

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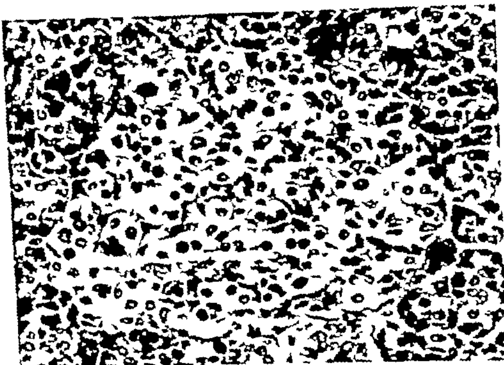


Fig. 13
Hydropic B-cells in a recent-onset juvenile diabetic. Chromium hematoxylin-phloxine. x 620

the disappearance of the B cells and, perhaps also, as a result of islet inflammation. In elderly patients, diabetic or not, ischemia, due to vascular sclerosis and occasionally pancreatitis, are mainly responsible for this feature. Lazarus and Volk (1962) attribute an important role to insular fibrosis in the pathogenesis of maturity-onset diabetes. According to their theory, the fibrosis would constitute an anatomic barrier between the B cell and the circulation and could therefore account for the sluggish insulin secretion, characteristic for maturity-onset diabetes. The absence of diabetes in numerous patients with severe degree of vascular sclerosis and islet fibrosis is not in accordance with this view.

Hyalinosis. Hyalinosis of the islets consists of a deposition of hyaline substance between the capillaries and the islet cells. As this accumulation increases in size, it compresses the islet cells, which may finally completely disappear (Fig. 15). Although islet hyalinosis was first described in a young diabetic (Opie, 1901), it appeared later on that this lesion is rare in juvenile diabetics, but that it is present in over 40 % of diabetics over 40 years of age. It is also found, albeit rarely, in non diabetics.

The nature of islet hyalin has not been completely clarified as yet. Many authors have stressed the similarity in staining reactions of amyloid and islet hyalin (Gellerstedt, 1938, Van Beek, 1939; Ahronheim, 1943; Arey, 1943; Ehrlich and Ratner, 1961; Porta, 196., Westermark, 1971, 1972, 1973 b, c). Under

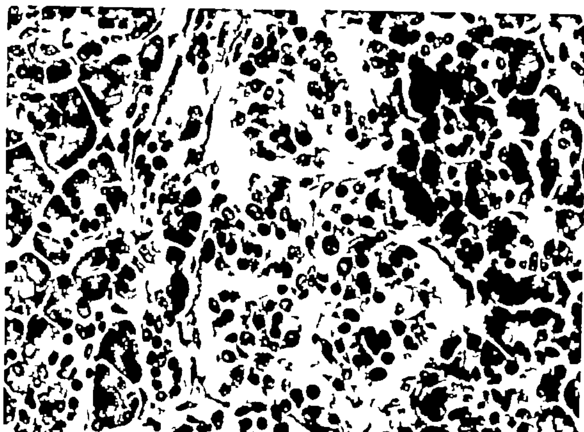


Fig 12
Nuclear polymorphism hyperchromatin and pycnosis in a recent-onset juvenile diabetic Chromium hematoxylin phloxine x 620

plete degranulation and an empty appearance of the cytoplasm (Fig 13) These changes are encountered in many B cells of young diabetics who died after a short clinical history whereas in elderly diabetics they affect only a few B cells and are extremely rare in non diabetics. Hydropic change has long been considered as a degenerative lesion till it was identified as the result of glycogen deposition (Toreson 1957) A simple glycogen deposition is usually not associated with damage of the cell organelles (Lazarus and Volk, 1962) It was also pointed out that a glycogen deposition should be distinguished from the so-called ballooning degeneration a truly degenerative change which was observed in dogs made severely diabetic by growth hormone The latter lesion is indeed characterized by a distension and vacuolization of the ergastoplasm In the pancreas of human diabetics, the distinction between the two types of changes is not always easy· however it is clear that both changes are secundary to hyperglycemia and are therefore devoid of etiological significance

Changes of the islet stroma

Fibrosis Fibrosis (Fig. 14) is the most common lesion in the islets of diabetics but it is not very specific being recognized as well in the islets of many elderly non diabetics (Gepts 1957 1965) In juvenile diabetics with a disease of long duration and in maturity-onset diabetics islet fibrosis is always associated with diffuse pancreatic fibrosis of the perilobular or interacinar type In juvenile diabetics islet fibrosis seems to result mainly from a collapse of the reticulin network after

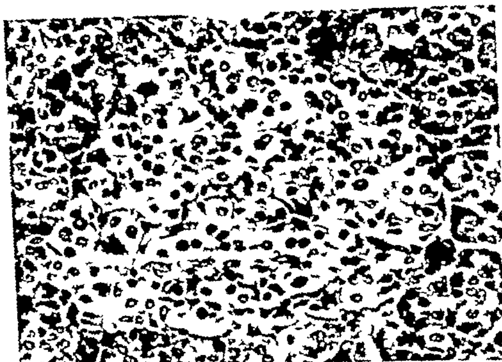


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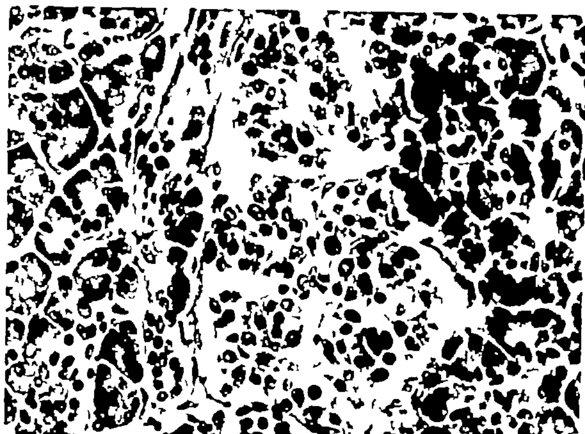


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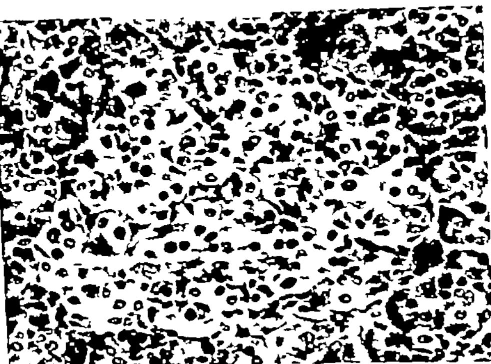


Fig. 13
Hydropic B-cells in recent-onset juvenile diabetic. Chromium hematoxylin-phloxine x 620

the disappearance of the B cells and perhaps also, as result of islet inflammation. In elderly patients, diabetic or not, ischemia, due to vascular sclerosis, and occasionally pancreatitis, are mainly responsible for this feature. Lazarus and Volk (1962) attribute an important role to insular fibrosis in the pathogenesis of maturity-onset diabetes. According to their theory the fibrosis would constitute an anatomic barrier between the B cells and the circulation, and could therefore account for the sluggish insulin secretion, characteristic for maturity-onset diabetes. The absence of diabetes in numerous patients with severe degree of vascular sclerosis and islet fibrosis is not in accordance with this view.

Hyalinosis. Hyalinosis of the islets consists of a deposition of a hyaline substance between the capillaries and the islet cells. As this accumulation increases in size, it compresses the islet cells, which may finally completely disappear (Fig. 15). Although islet hyalinosis was first described in young diabetic (Opie, 1901), it appeared later on that this lesion is rare in juvenile diabetics, but that it is present in over 40 % of diabetics over 40 years of age. It is also found, albeit rarely in non diabetics.

The nature of islet hyalin has not been completely clarified as yet. Many authors have stressed the similarity in staining reactions of amyloid and islet hyalin (Gellerstedt, 1938 Van Beek, 1939- Ahronheim, 1943 Arey 1943 Ehrlich and Ratner 1961 Porta, 1962 Westermark, 1971 1972, 1973 b c). Under

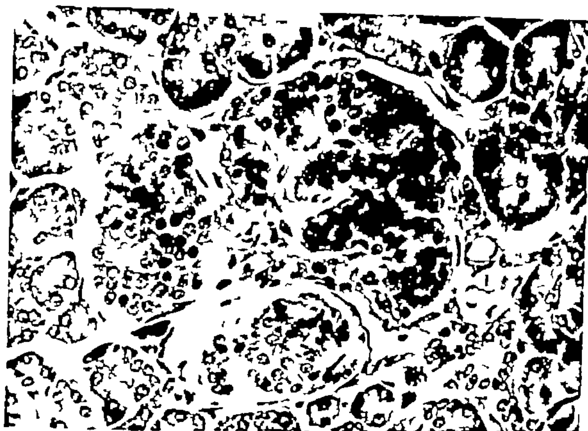


Fig 14
Islet fibrosis in a maturity-onset diabetic The darker cells are well granulated B-cells Chromium hematoxylin-phloxine x 620

the electron microscope islet hyalin has the same appearance as amyloid since it is composed of non branching 100 Å thick fibrils (Porta et al 1962 Lacy 1964) On the other hand *histochemical differences have been* pointed out by other authors acid mucopoly saccharides (Rinehart *et al* 1954) and lipids (Hartroft 1956) were found in islet hyalin but not in amyloid whereas tryptophan a typical constituent of amyloid could not be demonstrated in islet hyalin (Westermark, 1973 b c)

The origin of islet hyalin remains unknown It appears unlikely that it would result from a hyalin transformation of a pericapillary fibrosis or from an intrainsular extension of arte *riolar hyalinosis as suggested by Moschco*vitz (1951 1956) and by Lazarus and Volk (1962) because islet hyalin has completely different histochemical and ultrastructural characteristics The fact that islet hyalinosis is rare in juvenile diabetics is difficult to con*ciliate with Opie s view (1901) that it would* represent a product of B-cell degeneration *The close contact between the fibrils and the* B cells at the ultrastructural level with a definite orientation of these fibrils perpendicular to the cell surface and their presence in membrane limited pockets or channel inside the B cells strongly suggest that islet hyalin could be a product of B cell secretion (Lacy 1964 Westermark 1973 b c) The observation by Meisner (quoted by Warren et al 1966) that hyalin is usually present in functional adenoma but non in non-functional ones *supports this theory* An insulin immunoreactivity appears to be present in islet hyalin

(Westermark, 1973 b c) but this characteristic could only be elucidated after alkali treatment, which might explain the negative reaction obtained by Lacy (1964) Pearse et al. (1972) suggested that islet hyalin contains the C-peptide which represents a splitting product of the proinsulin molecule However it should be pointed out that hyalin deposits also occur in other polypeptide secreting endocrine tumours. Therefore the elucidation of the histogenesis of islet hyalinosis might be of importance for our general comprehension of the physiological and pathological aspects of polypeptide synthesis and secretion.

It appears doubtful that islet hyali could account for the secretory inertia of the B cells in elderly diabetics. This lesion is indeed lacking in more than 50 % of these diabetics and, when present affects only part of the islets.

Inflammatory Infiltration The inflammatory infiltration of islets which von Meyenburg (1970) adequately named "insulitis" has already been described at the beginning of this century when the first systematic studies on the pancreas in diabetics were performed (Opie 1901 Schmidt, 1902 Cecil 1909 Weichselbaum, 1910 Heiberg, 1911). They considered this lesion as rare although they admitted that it occurs more frequently in diabetic children. It is true that in elderly diabetics only two examples of insulitis have been reported so far (LeCompte and Legg, 1972) and that it has never been observed in those young diabetics in whom the disease has existed for more than one year (Gepts, 1965 Warren et al 1966) But in juvenile diabetics who have died within six months after the first symptoms of the disease insulitis is present in 2/3 of the cases (Gepts, 1965). Many isolated reports have confirmed the



Fig 15
Severe islet hyalinosis in maturity-onset diabetic Chromium hematoxylin-phloxine x 450

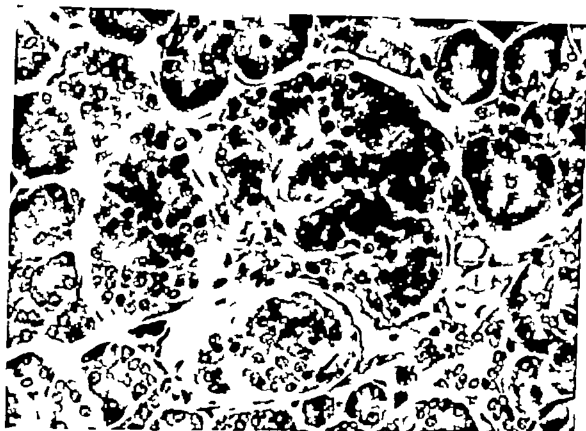


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The inflammatory infiltrate of insulitis is mainly composed of lymphocytes with occasionally a few polymorphonuclears and histiocytes (Figs 10 & 11) The proportion of islets involved is quite variable from very few to almost all islets Some of the affected islets are atrophic and fibrotic others are still large and contain well recognizable and functionally active A and B-cells (Gepts 1965)

Insulitis has arisen considerable interest in recent years because it might offer a clue to the etiology of the early-onset type of diabetes (LeCompte 1958 LeCompte et al 1966 Freytag 1973 Gepts 1976) In view of the reported temporal association of some viral diseases such as mumps (Gundersen 1927 Cole 1934 Kremer 1947 Melin and Ursing 1958 Hinden 1962 MacCrae 1963 Messaritakis et al 1971 Dacou-Vouretakis et al 1974) rubella (Forrest et al 1969 1971 Menser et al 1974) coxsackie B4-infections (Gamble and Taylor 1969 Gamble et al 1973) with the onset of diabetes a viral etiology has been suggested An immunopathological mechanism has also been considered because of the occasional coexistence of insulin-dependent diabetes with other diseases of accepted or presumed auto-immune pathogenesis such as pernicious anemia auto-immune thyroiditis auto-immune Addison s disease (Carpenter et al 1964 Ungar et al 1968 Gharib and Gastineau 1969 Hung et al 1970 Irvine et al 1970 Yoo and Kozak 1974 Nerup 1974 a) The histological characteristics of insulitis are compatible with both of these two hypotheses which moreover are not mutually exclusive

Insulitis has been reproduced experimentally through a viral inoculation (Craighead, 1966 1968 1971 1974 Müntefering, 1971 1972, 1974 Levy and Notkins 1971 Burch et al. 1971 1972 Wellman et al 1972 Boucher and Notkins 1972 Coleman et al 1973 Gamble and Taylor 1973 Kromann et al 1974 Hayashi et al 1974) as well as via immunological methods (Renold et al 1964 1969 LeCompte et al 1966 Toreson et al 1968 Lee et al 1969 Freytag et al 1968 1969 1972 1973 Federlin 1971 Klöppel et al 1971 1972 Nerup et al 1973 a Heydinger and Lacy 1974)

Direct evidence for an autoimmune process in the endocrine pancreas of insulin-dependent diabetics has been reported recently by Nerup et al (1971 1973 b 1974 b) MacCuish et al (1974 a b) Botazzo et al (1974) Lendrum et al. (1975) MacLaren and Huang, (1975) However it should be pointed out that the presence of circulating autoantibodies is no longer considered as an absolute proof for the autoimmune nature of a disease as such antibodies may be part of the normal and active state of the regulation of auto-immunity (Stiller et al 1975) According to our present concepts self-tolerance is the result of an active immune mechanism which can be disrupted by various factors (Heremans 1974) among which viruses occupy a prominent place

The frequent association of auto-immunity with an inherited immune deficiency (Fudenberg 1971) is interesting to point out in relation with the well-known genetic predisposition to diabetes. Recently several studies (Singal and Blajchman 1973 Nerup et al

1974 c; Cudworth and Woodrow 1974 Menser et al. 1974 Nelson et al 1975) have revealed an increased incidence of the HL-A8 and to a lesser extent of the W15-antigens of the HL-A histocompatibility system in insulin-dependent diabetics. It has been suggested that the occurrence of these antigens is associated with an increased susceptibility to certain viral infections, either as a direct effect or by a defective immune response.

3 Quantitative changes

Quantitative studies of the islet tissue are faced with technical difficulties due to its dispersion in a large exocrine gland of which it represents only 1 to 2 % in volume. Despite the fact that only crude methods could be applied converging results have been obtained by different investigators.

Numerical changes of the islets

All authors who have paid attention to this problem (see Kraus, 1929 Gepts, 1957 Warren et al. 1966) agree that diabetics generally have fewer islets than non-diabetics. This numerical reduction is much more pronounced in young than in elderly diabetics. The same conclusion has been reached regarding the *proportion of islet tissue* which is usually lower in the pancreas of diabetics than in non-diabetics. Again, this reduction is much more marked in young diabetics than in elderly diabetics. In the latter there is a considerable overlapping with values obtained in non-diabetics.

Total mass of islet tissue

The total mass of islet tissue is less in diabetics than in non-diabetics (MacLean and Ogilvie 1955 1959 Gepts, 1957 1965). In the case of elderly diabetics this reduction is of the order of 50 %. It is much more pronounced in juvenile diabetics, especially in those in whom the disease has been recognized for more than one year.

Cellular composition of the islets

Following Ferner (1952), numerous authors (see Gepts, 1957 Seifert 1959 Lazarus and Volk 196.., Warren et al 1966) have confirmed that the relative proportion of A-cells is usually increased in the islets of maturity-onset diabetics. However this increase is not specific because it occurs also in the islets of many non-diabetics. Furthermore it does not reflect a true hyperplasia of the A-cells, but results from a decrease in the number of B-cells. In the islets of juvenile diabetics an exact identification of the islet cells is extremely difficult, because many of these cells have an atrophic appearance B-cells even when almost completely degranulated, can still be recognized in those patients who have died less than six months after the clinical onset of the disease. Their number does, however not exceed 10 % of the normal values. In young diabetics who have survived for a longer time B-cells are rare or completely absent. Little information is available as yet on quantitative changes in the population of A (or D-) cells in the islets of diabetics. Fujita (1968) has reported an increased number of these cells in the islets of elderly diabetics. The application of immuno-histological techniques on the pancreas of two chronic juvenile diabetics has demonstrated the presence of glucagon-secreting A-cells and somatostatin-secreting D-cells in the islets B-cells could not be detected (Rufener et al. 1975).

Total mass of A and of B-cells

Despite the increased proportion of A-cells in the islets of diabetics, their total mass is not larger than in non-diabetics (MacLean and Ogilvie 1955 Gepts 1957). On the other hand the total mass of B-cells is decreased in all elderly diabetics on the average to about 45-50 % of the normal value In juvenile diabetics a determination of the total mass of B-cells and of the other islet cell components is hindered by their difficult identification, and even more so by the marked heterogeneity of the pancreas in these patients. From the reduced proportion of islet tissue and the marked decrease in the number of B-cells, it can be concluded that at the moment of clinical onset of the disease, a

occurence of lymphocytic infiltration in the pancreatic islets of young diabetics with a disease of short duration (Nagler and Taylor 1963 Crome et al 1967 Skiner 1967 Warne 1973 Gladish et al 1975) But the exact frequency of insulitis is still controversial. Ogilvie (1964) reported it as rare and Doniach (1973) did not find it once in 13 acute juvenile diabetics Recently Egeberg et al (1976) observed it in six among twelve recent-onset juvenile diabetics It has also been described in an infant with cytomegalovirus infection (Hultquist et al 1973) and in spontaneously diabetic animals namely in cows (Christensen and Schambye 1950 Barboni and Manocchio 1962 Pedini et al 1962) and in a cat (Gepts and Toussaint 1967)

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Conclusion

The endocrine pancreas exerts its key metabolic functions through multiple functional units which are dispersed over the entire pancreas. These units the islets of Langerhans, have been the subject of many morphological dissections, in search for the mechanisms responsible for normal and abnormal glucose homeostasis. At least four different islet cells have so far been distinguished all of which exhibit the ultrastructural features of secretory cells, the hormones insulin glucagon and somatostatin have been identified respectively in the B A and D cells, whereas the presence of gastrin remains doubtful. The secretory activity of islet cells appears highly dependent on various nutrients, gastro-intestinal hormones and neurotransmittors as could also be predicted by their location on the entero-portal axis and by their autonomous innervation The junctional complexes between the islet cells furthermore suggests the existence of internal control factors capable of (a)synchronizing the release of the islet polypeptides

The mechanisms through which extracellular factors govern the intracellular events in the endocrine pancreas remain poorly defined, although the stimulus-secretion coupling of the B-cell has received considerable attention In analogy to other tissues, membrane receptors regulate also the cyclic AMP levels which modulate significantly the B-cell functions. In addition, the B-cell is equipped with glucoreceptor the nature and the localization of which is still controversial. The glucose recognition represents an important physiological event for the B-cell crucial for the insulinotropic effect of other agents and capable of inducing hormone synthesis, transport and release. The newly synthetized proinsulin molecules are conveyed from the endoplasmic reticulum to the Golgi complex where the conversion to insulin is initiated parallel to the packaging of the secretory products into secretory vesicles. An intracellular organization of the secretory products can be proposed, which can be responsible for a non-random secretory pattern. The vectorial hormone transport to the plasma membrane might require the active participation of cytoplasmic microtubules which get assembled by insulinotropic agents. Both tubulin assembly and tubulin synthesis might be regulated by the islet adenylcyclase activity indicating a possible mechanism for the well known modulating effect of cyclic AMP upon insulin release. The role of calcium might on the other hand be more intimitaly involved with the emiocytotic process

An impairment in the secretory response to glucose appears a characteristic feature of maturity-onset diabetes which indicates that possible defect in the glucose recognition and/or the secretory proces of the B-cell might represent the pathogenic factor in this disease. It is most likely that various circumstances are capable of initiating a glucose intolerance associated with a deficient B-cell population. Such diverse pathogenic agents might affect the B-cell at different levels, which can explain the varying morphologic appearance of the endocrine pancreas in diabetic states. Both atrophic and hypertrophic islets composed of few or several granulated or degranulated B-cells have been described in human diabetes. No pathognomonic features can so far be identified with diabetic islets, although it is generally accepted that islet hyalinosis is frequently observed in elder diabetics this characteristic does not seem to cause the secretory defect but appears to correspond to an accumulation of secretory products. In juvenile diabetes an inflammatory infiltration of the islet tissue constitutes a common finding and might be related to the etiology of the disease Genetic factors may play a part either by predisposing to some types of viral infection or by inducing autoimmune reactions. Only in young diabetics

young diabetic depends on less than 10 % of the normal B cell population whereas B-cells have almost completely disappeared one year later (Gepts 1965) It should be pointed out however that exceptions may occur and that some young diabetics are able to maintain a significant although lower number of B-cells Furthermore the progressive disappearance of the B-cells from the pancreas of young diabetics does not necessarily progress in a steady way Histological observations suggest that it may be interrupted by spurts of local neoformation of islet cells

4 Histochemical studies

Slide histochemistry and biochemical studies of microdissected islet tissue have been performed on the pancreas of human diabetics and non-diabetics For obvious ethical and technical reasons such studies meet with considerable difficulties In the few studies reported until now (Gepts et al 1970 Gepts and Gregoire 1971) no significant differences could be detected between the islets of diabetics and those of non-diabetics regarding the activity of lactic dehydrogenase pyruvate kinase glucose-6-phosphate dehydrogenase glutamate oxalacetate transaminase and acid phosphatase A small but statistically significant ($p < 0.05$) decrease in the activity of isocitric dehydrogenase was observed in the islets of maturity-onset diabetics but its biological significance remains doubtful

On the other hand it has been possible to demonstrate with the aid of a radioimmunoassay of microdissected islet tissue that the B-cells of elderly diabetics contain only 50 % of the normal amount of insulin (Gepts et al 1970 Gepts and Gregoire 1971) This decrease was found in treated as well as in non-treated diabetics It appears unlikely that this reduced insulin content is related to an increased insulin output resulting from hyperglycemia In babies from diabetic mothers the pancreas contains normal insulin levels in spite of a tremendously increased insulin secretion demonstrating the ability of normal B-cells to compensate for an increased secretory rate In elderly diabetics the reduced amount of insulin in the B-cells probably reflects a functional incapability of these cells as compared to normal islet cells

5 Ultrastructural studies

Taking into account the requirements for an adequate fixation, it is understandable that very few studies have been performed on the ultrastructural changes of the islets in diabetics (Lacy 1964 Kawanishi et al. 1966, Yamada 1968 Kohama et al 1969) Lacy (1964) found no difference between the B-cells of diabetics and non-diabetics Kawanishi et al (1966) described an increase in lipids a decreased number of ribosomes and a small sized Golgi-apparatus in the B-cells of three maturity-onset diabetics These ultrastructural findings also point to a diminished functional activity of the B-cells in elderly diabetics.

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the reduction in the number of B-cells is sufficient to account for the secretory defect No clear-cut quantitative or qualitative changes have so far been described in the A D and D₁ cells of diabetic islets but the recent findings on the regulatory role of glucagon and somatostatin in normal and diabetic states certainly stress the possibility that diabetes might be a disease of the islet and not merely one of the B-cell

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Chemical and biological aspects of insulin and proinsulin

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Introduction

Although diabetes in man is well documented in ancient Egyptian, Oriental and Greek medical records (Papaspyros, 1964), it was not until the latter half of the 19th century that insight into the origin of this condition began to develop. At the close of the 19th century the classical experiments of Von Mering and Minkowski (1890) clearly demonstrated that the pancreas plays an important role in the prevention of diabetes. Over the ensuing 20 years many unsuccessful or only partially successful, attempts were made to isolate the anti-diabetic principle believed to exist in the pancreas but it wasn't until Banting and Best in 1921 ligated the exocrine duct in dogs allowing the destructive acinar tissue to atrophy that potent preparations of insulin could regularly be prepared. The name insulin was based on the appreciation, through the work of Opie that the hormone was derived from the islets of Langerhans within the pancreas. Actually the name insuline had been suggested as early as 1909 by deMayer and later again by Sir Edward Sharpey-Schafer. Preparative methods based on those of Banting and Best were rapidly adapted for the commercial preparation of insulin and by 1922 the first human patients began to receive injections of the lifesaving hormone.

But insight into the chemical nature of insulin was slower in coming. Although the fact that it was destroyed by the exocrine proteolytic enzymes suggested that it might be a protein, it was not at first appreciated that proteins could produce such dramatic biological effect as the lowering of blood sugar and the enhancement of carbohydrate utilization. When J.J. Abel (1926) first succeeded in crystallizing insulin considerable controversy surrounded the question as to whether the crystals of proteinaceous material actually contained the active biological principle or were merely its carrier (Murnaghan and Talalay 1967). In the 1930's little was known about the chemical nature of insulin aside from the most rudimentary knowledge of its amino acid composition (Jensen, 1938). Svedberg (1931), using the ultracentrifuge had determined the correct molecular weight of an insulin hexamer i.e. 35 100 and Jensen and Evans (1935) had succeeded in identifying phenylalanine as an N-terminal residue. When Sanger undertook to determine the complete structure of insulin and succeeded in doing so by the mid-1950's it was with the objective of showing that proteins did indeed have a defined amino acid sequence that was amenable to investigation (Sanger 1959). His brilliant success with insulin became one of the important milestones in the development of the new field of molecular biology. In truth over the years since 1921 insulin has been instrumental as a model protein in the developement of the entire field of protein chemistry first in the crystallographic studies of Abel and later of Scott and Fisher (1935) and Schlichtkrull (1958), and then in the structural studies of Sanger.

In the last decade insulin has one again served as a model for the complete chemical synthesis of a protein. This remarkable feat was accomplished almost simultaneously in laboratories in Germany (Zahn, 1965), Peking (Kung, et al. 1965) and the United States (Katsoyannis and Tometsko, 1966). These workers all used techniques in which separately synthesized A and B chains were combined under *oxidizing* conditions to yield biologically active insulin. The subsequent discovery of *proinsulin* (Steiner and Oyer 1967) demonstrated that the natural mechanism of insulin synthesis employs the inherent ordering ability of a single chain precursor form to facilitate the formation in high yield of the correct disulfide bonds of the hormone (Steiner and Clark, 1968), and it in turn has generated promising new approaches to the chemical synthesis of insulin (See Section VII). Yet another approach to this problem has resulted in the recent successful synthe-

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sis of human insulin by a Swiss group of scientists (Rittel et al 1975) In their procedure the A and B chains are built up on a starting peptide (A_{20-21} B_{17-20}) in which the A_{20} and B_{19} cysteines are already combined in disulfide linkage The chains are then extended by the imaginative use of selective blocking agents and the remaining disulfide bonds are formed unambiguously This synthesis represents a landmark in insulin synthetic studies in that it provides the final necessary synthetic confirmation of the correctness of the disulfide bond arrangement shown in the classical Sanger structure

Studies of the metabolic aberrations of diabetes and the effects of insulin on these have served as important stimuli to the development of the field of intermediary metabolism and its many important clinical applications in the study and treatment of inherited metabolic diseases But despite the many advances that have occurred in recent years in our understanding of the formation and secretion of insulin as well as of its physiological effects we still seem to be far from a complete understanding of the molecular details of its mechanism of action. Likewise our understanding of why diabetes afflicts many humans as well as a large variety of animal species is still far from complete This chapter will atempt to selectively review the present state of our knowledge concerning the functional organization of the insulin producing beta cells of the islets of Langerhans the chemistry and biology of the insulin molecule and its precursors and some current theories regarding its mechanism of action We will attempt to indicate how alterations in these or other areas may be related to the development of diabetes in man

Isolation, properties, and structure of insulin

1 Isolation and Characterization

Insulin occurs throughout the ertebrate kingdom, and mmunological and biological evidence indicates its presence in the digestive ystems of several invertebrate species including molluscs and echinoderms (Falkmer et al. 1973). The early recognition that ethanol or acid-ethanol extraction of pancreas inhibited proteolytic destruction of insulin has provided the basis for most modern preparative procedures (H mbel et al 1972) Acid-ethanol also efficiently extracts proinsulin, C-peptide and glucagon from islet tissue in most species. The acid-ethanol extract are partially purified by fractional precipitation and isoelectric precipitation with organic solvent to solubilize fats, and are then further resolved by gel filtration (Davoren, 196.) and, in some cases,by ion exchange chromatography (Chance et al. 1968 Steiner et al 1968). In our experience salting-out from acidic solutions is not effective for recovering small amounts of insulin and may lead to significant losses of C-peptide (Tager et al. 1975) For most analytical as well a preparative purposes it is usually preferable t omit acid salting-out steps and to proceed directly to gel filtration, convenient and highly reproducible method that separates proinsulin from insulin and thus permits pecific immunoassays to be made for insulin C-peptide and proinsulin, as well as for glucagon and its precursors A suitable flow sheet for extraction of pancreas of most mammals is shown in Fig. 1

Yields of insulin vary dependent on the source: for mammalian pancreas, 10-15 µmoles/kg wet weight, for fetal calf pancreas, 60-70 µmoles/kg: for fish islets, 300-500 µmoles/kg; for isolated rat islets of Langerhans 3 mmoles/kg.

With modern methods of purification, the biological activity of most mammalian insulin preparations ranges from 26-30 international units/mg. The bovine insulin standard of the International Union of Pure and Applied Chemistry is stated to have an activity of 25 I U /mg dry weight (Humbel et al. 1972) Although crystallization with zinc is a powerful method for purification of insulin it is now generally recognized that even repeated crystallization does not eliminate all impurities from insulin. Most crystalline preparations contain glucagon desamido insulin proinsulin and intermediate cleavage forms, ethyl esters of nsulin, dimers of insulin and larger aggregates of insulin and proinsulin with unknown components Gel filtration of crystalline preparations separates these component into essentially three fractions *a* component- material of high molecular weight eluting essentially in the void volume .e aggregates, *b* component-proinsulin, intermediate cleavage forms and insuli dimers component-insulin-like components including desamido forms arginyl insulins (insulin with arginine residues at B_{31} or at B_{31} and B_{32}) glucagon, and C-peptide. Further purification of the insulin-containing fractions by ion exchange chromatogr phy lug urea-containing buffers or 60 % ethanol as dispersing agents yields preparations that are better than 99% pure (i.e. the "mono component" or single component" insulins now being offered commercially These preparations are apparently much less antigenic than crystalline insulin preparations and show promise for therapeutic application (Bruni et al 1973 Deckert and Paulsen this volume).

The a say of insulin like most other protein hormones has always presented difficulties with regard to precision and sensitivity The various in h o blood sugar-lowering a says

sis of human insulin by a Swiss group of scientists (Rittel et al 1975) In their procedure the A and B chains are built up on a starting peptide (A_{20-21} B_{17-20}) in which the A_{20} and B_{19} cysteines are already combined in disulfide linkage The chains are then extended by the imaginative use of selective blocking agents and the remaining disulfide bonds are formed unambiguously This synthesis represents a landmark in insulin synthetic studies in that it provides the final necessary synthetic confirmation of the correctness of the disulfide bond arrangement shown in the classical Sanger structure

Studies of the metabolic aberrations of diabetes and the effects of insulin on these have served as important stimuli to the development of the field of intermediary metabolism and its many important clinical applications in the study and treatment of inherited metabolic diseases But despite the many advances that have occurred in recent years in our understanding of the formation and secretion of insulin as well as of its physiological effects we still seem to be far from a complete understanding of the molecular details of its mechanism of action Likewise our understanding of why diabetes afflicts many humans as well as a large variety of animal species is still far from complete This chapter will atempt to selectively review the present state of our knowledge concerning the functional organization of the insulin producing beta cells of the islets of Langerhans the chemistry and biology of the insulin molecule and its precursors and some current theories regarding its mechanism of action We will attempt to indicate how alterations in these or other areas may be related to the development of diabetes in man

preparation should include measurements of biological potency with isolated fat cells or liver cells as well as measurements of binding characteristics and full characterization of the protein in terms of its molecular weight, composition, homogeneity and if possible amino acid composition and sequence. For more detailed information regarding the physicochemical properties of insulin several recent reviews are recommended (Humbel, et al., 1972, Klostermeyer and Humbel 1966 Tager and Steiner 1974)

2. Insulin structure

The determination of the primary structure of bovine insulin (Fig. 2) by Sanger and his associates (Ryle, et al. 1955) provided the first known protein structure and it also led to rest the prevalent notions that proteins were not defined chemical entities. This demonstration thus provided an important corner stone of molecular biology which led to the recognition of the existence of the genetic code. The primary structures of insulins from more than 20 vertebrate species have been determined in the interval since the pioneering studies of Sanger and coworkers (Dayhoff 1973 Humbel, et al. 1972 Smith, 1972a, Peterson et al. 1975). These results summarized in Fig. 3 indicate that amino acid substitutions can occur at many positions within either chain without greatly altering the biological effectiveness of the hormone as measured in various bioassay systems. On the other hand certain structural features are conserved throughout vertebrate evolution including the positions of the 3 disulfide bonds the N-terminal and C-terminal regions of the A chain and the hydrophobic residues in the C-terminal region of the B chain, as well as others. Since chemical modifications in any of these regions tend to markedly reduce or abolish biological activity these evidently play important roles in maintaining important secondary and tertiary structural features needed for biological activity (Humbel, et al. 1972 Carpenter 1966). The C-terminal hydrophobic sequence of the B chain (residues 23-27) also plays an important role in the formation of insulin dimers as described below.

As might be anticipated from the extensive amino acid substitutions that occur between mammalian and piscine insulins it is not surprising that the immunological cross-reactivity between these proteins is rather weak. Generally very low cross-reactivity can be detected by means of conventional immunoassays, especially when the heterologous insulin is used as the labeled tracer. For detailed considerations of insulin antigenicity in relation to its structure several recent reviews are recommended (Humbel et al. 1972 Arquilla, et al. 1972).

Recently we have isolated a primitive insulin from the islet organs of the Atlantic hagfish, Myxine glutinosa (Peterson et al. 1975). We have determined the amino acid sequence of this cyclostomian hormone by Edman degradation of the S-carboxymethylated and oxidized A and B chains, and of various tryptic peptides derived from the chains. The 52 residue hagfish insulin has many structural features in common with other vertebrate insu-

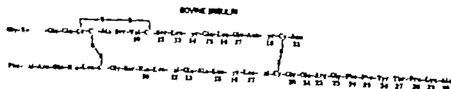


Fig.
Covalent structure of bovine insulin (Sanger 1958)

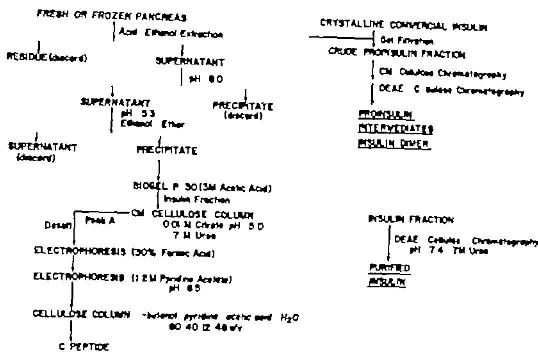


Fig 1
Flow diagram for the isolation of proinsulin insulin and C peptide from fresh pancreas or from commercial insulin preparations For methodologic details see Steiner et al (1968 1971)

that often are used by pharmaceutical houses require too much material to be very useful for most experimental laboratories When sufficient amounts of hormone are available (i e >0.5 mg) polyacrylamide gel electrophoresis with appropriate standards can provide a wealth of useful information regarding the homogeneity and quality of the preparations (Mirsky and Kawamura 1966 Chance et al 1968 Steiner et al 1968) This method also gives indications as to the state of amidation of the insulin or proinsulin which may reflect the harshness of the acid conditions applied in the extraction and this method also can reveal the extent to which autolysis may have occurred in the pancreas prior to extraction Other routinely used biochemical methods for assessing the purity of proteins are equally applicable of course but it must be borne in mind that even though all tests indicate that homogeneity has been achieved the biological activity of the preparation must be examined directly to ascertain that no chemical damage to the hormone has occurred

The recent introduction of hormone binding assays using isolated plasma membrane preparations promises to provide more sensitive and reliable methods for screening material for biological potency *in vivo* since these methods have thus far demonstrated a good correlation between binding and measured biological potency (Freychet et al 1971 Freychet et al 1974 Gliemann and Gammeltoft 1974) However both binding test as well as immunoassays can be misleading since neither necessarily measures the true biological effectiveness of the hormone Thus the thorough characterization of any insulin

preparation should include measurements of biological potency with isolated fat cells or liver cells as well as measurements of binding characteristics and full characterization of the protein in terms of its molecular weight composition, homogeneity and if possible, amino acid composition and sequence. For more detailed information regarding the physicochemical properties of insulin several recent reviews are recommended(Humbel et al., 1972 Klostermeyer and Humbel 1966, Tager and Steiner 1974)

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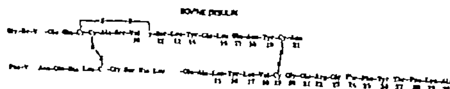


Fig. 2
Covalent structure of bovine insulin (Sanger 1958).

HAGFISH INSULIN – SEQUENCE COMPARISON

A Chain

	1				5					10					15					20	21
Hagfish	Gly	Ile	Val	Glu	Gln	Cys	Cys	His	Lys	Arg	Cy	Ser	Ile	Tyr	Asn	Leu	Gln	Asn	Tyr	Cys	Asn
Porcine	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn
Other Residues			His	Asp				Ala	Gly Arg Asn	Pro Val Thr		Asp Asn Thr	Arg Lys	Phe Hi	Asp			Ser			

B Chains

	0	1				5					10					15					20
Hagfish		Arg	Thr	Thr	Gly	H	Leu	Cys	Gly	Lys	Asp	Leu	Val	Asn	Al	Leu	Tyr	Ile-	Ala	Cys	Gly
Porcine		Phe	Val-	Asn-	Gln	His	Leu	-Cys	Gly	Ser	H	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly
Other Residues	Val Met	Ala	Pro Ala	Pr Lys Ser Ala	Arg Pro					Pro	Asn			Asp	Thr			Ser			Gln

	21				25					30	31
Hagfish	V l	Arg-	Gly-	Phe-	Phe	Ty	Asp	Pro	Thr	Lys	Met
Porcine	Glu	-Arg-	Gly	Phe-	Phe	Tyr	Thr	Pr	Lys	Ala	
Other Residues	A p	Asp					Ser Ile Gln	Ser	Met	Ser Thr Asp	(Arg)

Fig 3
The amino acid sequence of hagfish and porcine insulins with substitutions at each position in other known insulins shown for comparison

lins including the locations of the six half-cysteines the amino-terminal 7 residues and the carboxyl-terminal 6 residues of the A chain and several shorter sequences in the B chain that are known to comprise the dimer interface in porcine insulin crystals (Fig. 3) Of the 24 residues which are invariant among the other known insulins 23 are identical in hagfish insulin. However hagfish insulin differs from human insulin at 19 positions out of 51 and at 16 of these sites it contains residues not previously observed in vertebrate insulins The B chain also contains an additional carboxyl-terminal residue of methionine making it one residue longer than the usual 30 residue mammalian B chains

An interesting difference in hagfish insulin is the substitution of aspartic acid for histidine at position 10 of the B chain (Fig. 3) an important residue for zinc binding in the formation of insulin hexamers Hagfish insulin also lacks certain other structural features that have been shown by Hodgkin and coworkers to participate in the formation of hexamers (Blundell et al 197?) Nevertheless it crystallizes under conditions similar to those required for crystallization of mammalian insulin but in a different form (Fig. 4) and zinc or other divalent metal ions are not required (Peterson, et al 1974) The biological activity of hagfish insulin has been reported to be ? I U /mg (i e 8 % of mammalian insulin) as determined by the fat pad assay (Weitzel et al 1967) Further activity studies with other bioassay systems are in progress

The recent elucidation by Hodgkin and her coworkers of the three-dimensional structure



conservation in hagfish insulin of many primary structural features known to be concerned with the formation of secondary and tertiary structure. Taken altogether these results suggest that despite some variations in certain species such as the guinea pig and coypu (Smith, 197? a), the molecular structure of insulin as well as its tendency to form isologous dimers has remained remarkably con-

Fig 4
Crystals of hagfish insulin prepared in our laboratory by S O Emdin.

of porcine insulin initially at a resolution of 2.8 Å and with recent refinements at 1 9 Å represents an important breakthrough in the study of peptide hormone structure. The results have proven invaluable in interpreting much of the available chemical data on the properties of insuli (Blundell et al 1972). Detailed knowledge of the spatial organization of the molecule also promises to provide further insight into the molecular mechanism of binding and action of insulin. The hexameric unit of crystalline zinc insulin (Fig. 5) consists of three dimers arranged around a major three fold axis which passes through two zinc atoms, each of which is coordinated with the imidazole groups of three B histidine residues and located just above or below the plane of the hexamer (Blundell et al. 1971). The insulin dimers are held together in the crystals by hydrogen bonds between the peptide groups of residues 24 and 26 within the C-terminal region of the B chain, forming an antiparallel beta pleated sheet structure. The locations in space of the known invariant amino acid w thin the insulin monomer are show in F g 6

Preliminary x-ray diffraction studies on hagfish insulin crystals suggest a very similar arrangement of the molecular backbone in this ery primitive insulin (Cutfield, et al 1974). These results are consistent with the

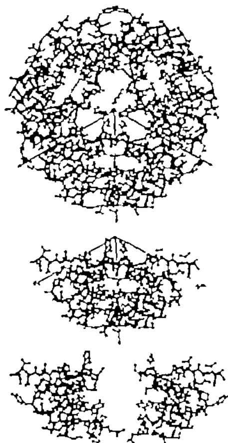


Fig 5
Th complete hexamer of porcine insulin showing the development of dimers from monomers and their organization into the hexamer (Reproduced with permission from Blundell et al 1972).

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Porcine	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys-	Asn
Other Residues			His	Asp				Ala	Gly	Pro		Asp	Arg	Phe	A			Ser			
									Arg	Val		Asn	Lys	His							
									Asn	Thr		Thr									

B Chain

	0	1				5					10					15					20
Hagfish		Arg	Thr	Thr	Gly	His	Leu	Cys	Gly	Lys	Asp	Leu	Val	Asn-	Ala	Leu	Tyr	Ile-	Ala	Cys	Gly
Porcine		Phe-	Val	Asn-	Gln	His	Leu-	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu-	Val	Cys	Gly
Other Residues	Val	Ala	Pro	Pro	Arg					Pro	Asn			Asp	Thr			Ser			Gln
	Met		Ala	Lys	Pro																
				Ser																	
				Ala																	

	21				25					30	31
Hagfish	Val	Arg-	Gly-	Phe	Phe	Tyr	Asp	Pro	Thr	Lys	Met
Porcine	Glu	Arg-	Gly	Phe-	Phe	Tyr	Thr	Pro	Lys-	Ala	
Other Residues	Asp	Asp					Ser	Ser	Met	Ser	(Arg)
							Ile			Thr	
							Gln			Asp	

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The recent elucidation by Hodgkin and her coworkers of the three-dimensional structure

Binding, action and degradation of insulin

Although insulin was first recognized as a powerful stimulus to the permeation and utilization of glucose by various sensitive tissues (Levine and Goldstein, 1955) it is now widely accepted that in addition to this antidiabetic or hypoglycemic action the hormone also strongly influences the biosynthesis as well as the breakdown of the major foodstuff i.e. carbohydrates, proteins, and lipids (Krahl 1961 Steiner 1966 Fritz, 1972 Steiner and Freinkel 1972) It is one of the preeminent regulators of anabolism in the organism, its presence being essential for the action of growth hormone Under normal conditions its actions are closely integrated with those of a number of other hormones that influence intermediary metabolism such as thyroxine (or triiodothyronine) growth hormone glucagon and glucocorticoids, resulting in the most efficient storage and/or utilization of the varying nutrients of the diet for body growth, energy production, thermogenesis and tissue maintenance The absence or excess of any of these important hormones may lead to severe derangements in the metabolic economy of the organism which are reflected in altered growth patterns, negative nitrogen balance and/or diabetes mellitus Detailed descriptions of these disorders are to be found elsewhere (see chapter of Luft-Cerasi this volume Williams, 1974). Nevertheless despite the relatively large volume of experimental and clinical data on the metabolic effect of insulin or of its deficiency state the molecular events underlying the action of the hormone remain obscure (Pilkis and Park 1974).

The possibility that insulin acts by binding to its target tissues was first suggested by Stadie et al. (1953) on the basis of the uptake of iodo-insulin by isolated rat hemidiaphragms *in vitro*. This interaction of insulin with muscle tissue was carefully analyzed by Wohltmann and Narahara (1966) using isolated frog sartorius muscles. They observed that washing at 0° did not remove bound hormone from the tissue but that enhancement of sugar permeability did not occur unless the tissue was warmed. They concluded that the effect of insulin on permeability appears to involve a temperature-dependent reaction that occurs after the initial binding of the hormone. Antoniades and Gershoff (1966) and Crofford (1968) demonstrated insulin uptake by isolated fat cells and attributed this rapid reaction to an association of the hormone with the plasma membrane

More recently the binding of iodo-insulin to receptors in the plasma membranes of its target cells has been directly demonstrated (House 1971 Kono and Barham 1971 Cuatrecasas, 1971 a, Freychet, et al. 1971) The correspondence between the relative biological potencies of various insulin analogues and their relative abilities to compete with iodo-insulin for receptor binding (Freychet, *et al* 1974 Gliemann and Gammeltoft 1974 Gammeltoft and Gliemann, 1973 Simon et al 1974) (see Fig. 7 and Table I) as well as the finding that trypsin treatment abolishes both iodo-insulin binding and the biological effects of the hormone (Kono and Barham, 1971 Kono 1969 Cuatrecasas, 1971 b El-Allawy and Gliemann, *1972*, *Fain* and Loken, 1969) suggests that the association between *insulin and its receptor is* the physiologically significant one However the finding that the concentration of hormone necessary for half maximal activation of glucose utilization *in fat* cells is much lower than the concentration necessary for 50 % inhibition of *binding of iodo-insulin* to fat cells and to fat cell plasma membranes (Kono and Barham, *1971*) suggests that the relationship between the initial binding and the final biological ef

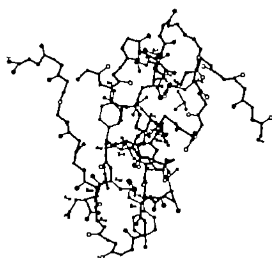


Fig 6
View of a porcine insulin monomer oriented perpendicular to the three-fold axis Only the side chains of known invariant residues are shown (Reproduced with permission from Blundell et al 1972)

stant throughout the evolution of the vertebrates The fact is further reflected in the relatively high interspecies crossover of biological potency among the known insulins i e most fish insulins are only slightly less active than mammalian insulins and as mentioned above hagfish insulin has been reported to have 5-10 % of the biological activity of bovine or porcine insulin in various mammalian test systems

There is a further implication in these findings that the receptor(s) for insulin also have undergone relatively little change throughout vertebrate evolution This conservatism of structure is similar to that seen among many other functional proteins including a variety of enzymes as well as the electron carrier protein cytochrome c Cytochrome c molecules from distant species all function remarkably similarly in mammalian mitochondrial preparations (Acher 1974) On this basis one might speculate that the structure of insulin and its receptor protein(s) do not simply represent arbitrary lock and key designs to subserve simple recognition purposes but rather that the insulin-receptor complex fulfills some specific chemical function in the plasma membrane either enzymatically as a catalytic unit or possibly as an intramembrane ionophore or translocase (Steiner 1966). The insulin receptor will be discussed in greater detail in the next section

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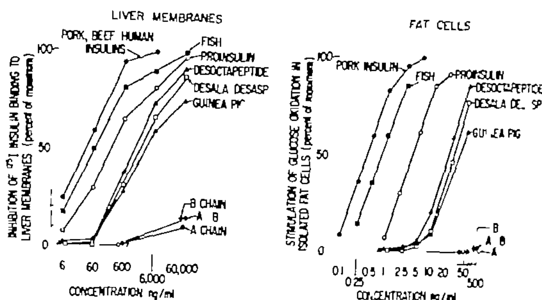


Fig 7
Comparison of effects of insulins and various insulin derivatives on porcine ¹²⁵I-insulin binding to liver membranes (left) and glucose oxidation in fat cells (right) (Reproduced with permission from Freychet et al 1971)

fect of the hormone is not a simple one This disparity between the Kd of binding and the Kd of effect has been rationalized by invoking either the existence of a large number of excess receptors with a uniform affinity for insulin (Kono and Barham 1971) or the existence of a small subpopulation of high-affinity binding sites responsible for biological activity (Gavin et al 1973 Kahn et al 1974 Hammond et al 1972)

It is not known whether the insulin receptor interaction is in itself sufficient to trigger the hormone response Although the observations of Cuatrecasas and others (Cuatrecasas 1969 Oka and Topper 1971 Blatt and Kim 1971 Tarui et al 1972) that insulin covalently linked to Sepharose beads or dextrans stimulates a variety of cells and tissues supports this concept the stability potency and biological characteristics of covalently linked hormone-polymer preparations have been questioned (Davidson et al 1972 a & b Katzen and Vlahakes 1973 Oka and Topper 1974)

The nature of the insulin receptor has for the most part been inferred from its behaviour in binding studies with the plasma membranes of liver and fat cells (Freychet et al 1971 Cuatrecasas 1974) as well as isolated thymocytes (Goldfine and Sherline 1972) lymphocytes (Gavin et al 1972) and nervous (Szabo Szabo 1972) and mammary tissue (O Keefe and Cuatrecasas 1974) The concomitant depression of iodo-insulin binding and of the stimulatory effect of insulin on glucose oxidation in fat cells by trypsin treatment (Kono and Barham 1971) suggess that the receptor is a protein whose recognition site is located on the exterior surface of the cell Cuatrecasas and coworkers have used detergents to isolate an insulin binding protein from liver cells and adipocytes (Cuatrecasas 1972) The protein appears to be asymmetric and has a molecular weight of about 300 000 (240 000) but no information is available regarding the nature of the constituent polypeptide chains For an excellent discussion of studies on membrane receptor proteins see the review by Cuatrecasas (1974)

Recent studies suggest that the insulin-receptor interaction in intact cells may not be a simple reversible equilibrium as has generally been thought (Gammeltoft and Gliemann, 1973 Kahn et al., 1974) The studies of S Terris in our laboratory have shown that the velocity of insulin degradation is proportional to the amount of [^{125}I]iodo-insulin bound over a wide range of insulin concentrations (Fig. 8) and that insulin binding and degradation velocity are depressed in parallel by mild pretreatment of the hepatocytes with proteases or in the presence of appropriate concent rations of several insulin analogues These findings suggest that receptor-bound insulin is the initial substrate for insulin degradation in hepatocytes and are compatible with some form of compartmentalization of the insulin binding and degrading sites. That is, insulin bound to a receptor located on the external surface of the cell may be transferred by pinocytosis or by some other form of transport to an intracellular or intramembranous degra ding site where rapid degradation occurs by a relati e exces of degradative enzyme( )

The 8-10 min lag in degradation which is observed following the initial binding of insulin to hepatocytes (Terris and Steiner 1975) may represent the time required for transfer of insulin from a recognition site to a degrada ti e site Compartmentalization could also account for the apparently identical specificities of the binding and degradative processes in intact cell (Terris and Steiner 1975) as opposed to the discrepancies between these parameters reported by other investigators who have studied cell membrane preparations (Freychet et al 1972) The observation of deMeyts et al (1973) that high insulin concentrations enhance the dissociation of iodinated insulin from lymphocyte receptors, as well s the close relationship between the binding and degradation of insulin in the liver would appear to provide plausible mechanisms for the regulation and termination of the biological signal of insulin. Further study of the receptor protein and its properties will be required to elucidate the molecular bases for these interesting phenomena. *Until* the molecular link between the receptor binding

Tabl 1 Th binding affinit of modified insulins t f t ell
(Reproduced from Gli mann nd Gammeltoft 1974)

Modified insulin and control insulin	KI in nM (with 95 % confidence limits)	Binding affinity % of insulin (mean)	Biological potency % of insulin
Des Phe[B1]	5.4 (4.2 7.0)	70 %	78- 102 %
Insulin control	3 7 (2 2 6 6)		
A1-monoacetyl	13.8 (9 8-19.3)	25 %	35- 40 %
Insulin control	3 4 (2.5- 4 8)		
A1 B29-diacetyl	12.2 (3 4-39 4)	23 %	26- 37 %
Insulin control	2.8 (1 6- 5 3)		
Des Gly	394 (181 1032)	0 6 %	0 4- 0 6 %
Insulin control	4 (1 3- 4.5)		
[Leu]	31 9 (22 9-43.8)	13 %	14- 18 %
Insulin control	4.1 (2.9- 6.1)		
A1 B29-suberoyl	137 (82 9-211)	2.5 %	1 9- 7 1 %
Insulin control	3.4 (2.1 5.8)		
A1-B29-dodecoyl	25 9 (16.7 39.2)	10 9 %	10- 13 %
Insulin control	2.8 (1 6- 5.3)		
B1 B1 PBC dimer	5.8 (4 4- 7 7)	42 %	34- 40 %
Insulin control	4 (1 9- 3.2)		
Despentapeptide B[26]	2.4 (15 7 37.6)	14.2 %	15- 18 %
Insulin control	3.4 (1 7 7 1)		

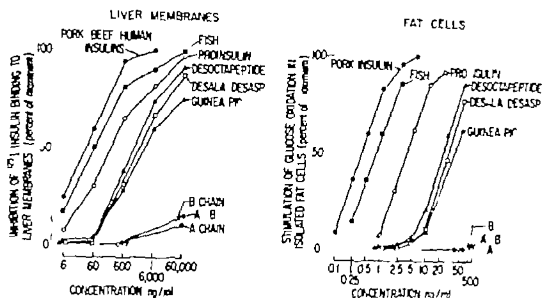


Fig 7
Comparison of effects of insulins and various insulin derivatives on porcine ^{125}I-insulin binding to liver membranes (left) and glucose oxidation in fat cells (right) (Reproduced with permission from Freychet et al 1971)

fect of the hormone is not a simple one This disparity between the Ki of binding and the Ki of effect has been rationalized by invoking either the existence of a large number of excess receptors with a uniform affinity for insulin (Kono and Barham 1971) or the existence of a small subpopulation of high-affinity binding sites responsible for biological activity (Gavin et al 1973 Kahn et al 1974 Hammond et al 1972)

It is not known whether the insulin receptor interaction is in itself sufficient to trigger the hormone response Although the observations of Cuatrecasas and others (Cuatrecasas 1969 Oka and Topper 1971 Blatt and Kim 1971 Taruı et al 1972) that insulin covalently linked to Sepharose beads or dextrans stimulates a variety of cells and tissues supports this concept the stability potency and biological characteristics of covalently linked hormone polymer preparations have been questioned (Davidson et al 1972 a & b Katzen and Vlahakes 1973 Oka and Topper 1974)

The nature of the insulin receptor has for the most part been inferred from its behaviour in binding studies with the plasma membranes of liver and fat cells (Freychet et al 1972 Cuatrecasas 1974) as well as isolated thymocytes (Goldfine and Sherline 1972) lymphocytes (Gavin et al 1972) and nervous (Szabo Szabo 1972) and mammary tissue (O Keefe and Cuatrecasas 1974) The concomitant depression of iodo-insulin binding and of the stimulatory effect of insulin on glucose oxidation in fat cells by trypsin treatment (Kono and Barham 1971) suggess that the receptor is a protein whose recognition site is located on the exterior surface of the cell Cuatrecasas and coworkers have used detergents to isolate an insulin binding protein from liver cells and adipocytes (Cuatrecasas 1972) The protein appears to be asymmetric and has a molecular weight of about 300 000 (?40 000) but no information is available regarding the nature of the constituent polypeptide chains For an excellent discussion of studies on membrane receptor proteins see the review by Cuatrecasas (1974)

Recent studies suggest that the insulin-receptor interaction in intact cells may not be a simple reversible equilibrium as has generally been thought (Gammeltoft and Gliemann 1973 Kahn, et al 1974) The studies of S Terris in our laboratory have shown that the velocity of insulin degradation is proportional to the amount of [^{125}I]iodo-insulin bound over a wide range of insulin concentrations (Fig. 8) and that insulin binding and degradation velocity are depressed in parallel by mild pretreatment of the hepatocytes with proteases or in the presence of appropriate concentrations of several insulin analogues. These findings suggest that receptor-bound insulin is the initial substrate for insulin degradation in hepatocytes and are compatible with some form of compartmentalization of the insulin binding and degrading sites. That is insulin bound to a receptor located on the external surface of the cell may be transferred by pinocytosis or by some other form of transport to an intracellular or intramembranous degrading site where rapid degradation occurs by a relative excess of degradative enzyme(s).

The 8-10 min lag in degradation which is observed following the initial binding of insulin to hepatocytes (Terris and Steiner 1975) may represent the time required for transfer of insulin from a recognition site to a degradative site Compartmentalization could also account for the apparently identical specificities of the binding and degradative processes in intact cells (Terris and Steiner 1975) as opposed to the discrepancies between these parameters reported by other investigators who have studied cell membrane preparations (Freychet et al. 1972) The observation of deMeyts et al. (1973) that high insulin concentrations enhance the dissociation of iodinated insulin from lymphocyte receptors as well as the close relationship between the binding and degradation of insulin in the liver would appear to provide plausible mechanisms for the regulation and termination of the biological signal of insulin. Further study of the receptor protein and its properties will be required to elucidate the molecular bases for these interesting phenomena. Until the molecular link between the receptor binding

Table 1 Th binding affinitie of modified insulins to fat cells (Reproduced from Gliemann and Gammeltoft 1974)

Modified insulin and control insulin	Ki in nM (with 95 % confidence limits)		Binding affinity % of insulin (mean)	Biological potency % of insulin
Des Phe[B1]	5 4	(4.2 7.0)	70 %	78- 102 %
Insulin control	3 7	(2.. 6 6)		
A1-monoacetyl	13.8	(9 8-19.3)	25 %	35- 40 %
Insulin control	3 4	(2 5- 4 8)		
A1 B29-diacetyl	12.2	(3 4-39 4)	23 %	26- 32 %
Insulin control	2 8	(1.6- 4.3)		
Des Gly	394	(181 1032)	0 6 %	0 4- 0.6 %
Insulin control	2.4	(1.3- 4 5)		
[Leu]	31 9	(22.9-43.8)	13 %	14- 18 %
Insulin control	4.1	(2.9- 6.1)		
A1 B29-suberoyl	137	(82.9- (1)	2.5 %	1.9- 7 1 %
Insulin control	3.4	(2.1 5 8)		
A1-B29-dodecoyl	25 9	(16 7 39.2)	10 9 %	10- 13 %
Insulin control	2 8	(1.6- 5.3)		
B1 B1 PBK dimer	5.8	(4 4- 7 7)	42 %	34- 40 %
Insulin control	2 4	(1 9- 3 2)		
Despentapeptide B[26]	4	(15 7 37 6)	14 2 %	15- 18 %
Insulin control	3 4	(1 7 7 1)		

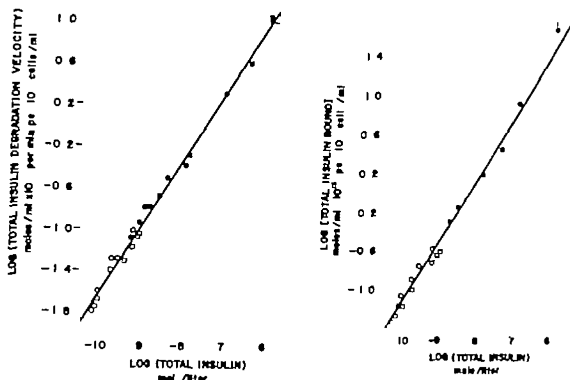


Fig 8
Concentration dependence of insulin binding (left) and insulin degradation velocity (right) in isolated rat hepatocyctes (Reproduced from Terris and Steiner 1975)

and the biological effects of insulin is clarified, the possibility remains that some of the hormone s effects may result from its translocation into the cell where active fragments may be generated Such a mode of action has recently been recognized in the case of the diphtheria toxin (Pappenheimer and Gill *1973) abrin and ricin (Olsnes and Pihl 1973* Refsnes et al 1974) and could also conceivably occur in the case of growth hormone where there are indications of an active core *that may be unmasked by limited proteolysis* (see Niall et al 1973) Also of interest in this connection is the recent work of Goldstein and Brown (1974) who have demonstrated *the specific binding of low density lipoprotein* (LDL) to cultured fibroblasts In their system binding led both to a biological effect i e inhibition of cholesterol biosynthesis and to the degradation of the LDL molecule

Much attention has recently been focused on the detailed mechanisms by which insulin activates the synthesis of glycogen lipids proteins and nucleic acids within cells as well as on its antilipolytic action and effects on various transport systems It is beyond the scope of this brief discussion to survey this important and large research area but these topics have been covered in detail in several recent reviews (Pilkis and Park 1974 Fritz 197? Steiner and Freinkel 197?)

Proinsulin and insulin biosynthesis

Although precursor or zymogen forms of a variety of enzymes most notably of proteases, have been known for many years it was only recognized that limited proteolysis might play more general role in protein biosynthesis after the discovery of proinsulin in 1967 (Steiner and Oyer 1967) Since then limited proteolysis has been found t occur in the formation of a variety of small peptide hormones as well as of many viral capsule proteins (Jacobson and Baltimore 1968 Kiehn and Holland, 1970) serum albumin (Geller et al. 1972 Judah et al 1973) and even connective tissue structural proteins such as collagen (Bornstein 1974). A distinctive feature of the process of insulin biosynthesis and one which sets it apart from classical zymogen activation is the intracellular proteolytic conversion of the precursor to the hormone prior to its storage and secretion from the beta cells (Steiner 1967) The intracellular localization and precise mechanism of th proteolytic process in the beta cell i problem of considerable interest from the point of view of the cell biologist This ystem also may prove seful as a model for the study of the biosynthesis of a number of important cellular constituents such as membrane-localized proteins, various intracellular organelles and perhaps even of some cellular enzymes (Steiner et al 1972).

1 Structure and properties of proinsulin

Proinsuli consists of single polypeptide chain ranging in length from 78 (dog) to 86 (human horse rat) amino acid residues (Steiner et al 1973 Chance et al. 1968). The ariations in length in the mammalian proteins occur only in the connecting polypeptide portion which links the carboxyl terminus of the insulin B chain to the amino terminus of the insulin A chain. The primary structure of bovine proinsulin (Steiner et al. 1968 Nolan, et al., 1971) is shown in Fig. 9 All the known mammalian proinsulins have pairs of basic residues at either end of the connecting peptide which link the connecting polypeptide to the insulin chains These residues are excised during the conversion of proinsulin to insulin and the resulting products are native insulin plus the remainder of the connecting polypeptide segment lacking amino- or carboxyl-terminal basic residues (Steiner et al. 1971) This peptide has been designated the C-peptide.

Despite its considerably larger molecular size proinsulin is remarkably similar to insulin in many properties, including solubility isoelectric point (Steiner et al. 1972) self-associative properties (Frank and Veros 1968) and reactivity with insulin antisera (Steiner et al 1969 Rubenstein et al. 1969 b 1970 b). These observations and a variety of preliminary evidence from other studies trongly suggest that the conformation of the insulin moiety in proinsulin is nearly identical to that of insulin itself (Steiner et al., 1972) It i of interest that the connecting peptide is much larger than would seem to be required to bridge the short 8 Å gap between the ends of the B and A chains (see Fig. 6). Although the connecting peptide may be folded over a portion of the surface of the insulin monomer it does not completely mask the "active site" since intact proinsulin still exhibits 3-5 % biological activity in several systems in vitro (Narahara, 1972 Gliemann and Sorensen, 1970). (It is unlikely that any significant cleavage or "activation" of proinsulin occurs in these tissues to account for this level of intrinsic activity (Lazarus et al., 1970 a) Similarly low but definite levels of biological acti ity have been observed in the case of several other peptide hormone precursors so that in thi respect, also these proteins differ from classical zymogen forms which are

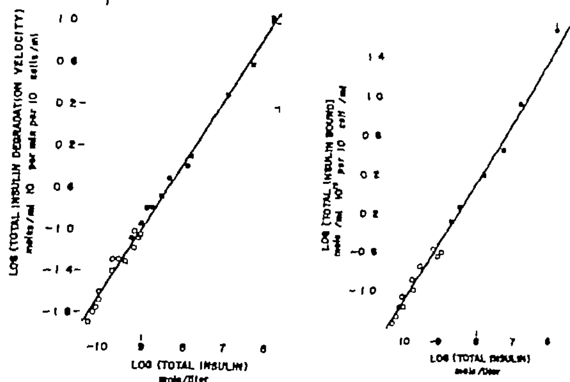


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In addition to the intact proinsulin, several intermediate forms have been identified in rat islets (Clark and Steiner 1969 Tager et al. 1973) These are partly cleaved forms of rat proinsulin which have two chains, as in insulin but which still retain the connecting peptide or fragment thereof attached to the A or B chain. During a "chase" incubation of islets labeled with ^{3}H-leucine radioactivity is transferred from these components as well as from proinsulin to insulin (Clark and Steiner 1969).

Comparative studies of insulin biosynthesis in the cod (Grant and Coombs 1971) and angler fish (Trakatellis and Schwartz, 1970) as well as in such primiti ertebrates as cyclostomes (Emdin et al. 1973) indicate the formation and cleavage of a proinsulin similar in size to the mammalian proteins A requirement for trypsin-like cleavage has been demonstrated for both of the fish proinsulins, and an interesting intermediate cleavage form having an N-terminal tripeptide A-chain extension, has been isolated from anglerfish islets by Yamaji et al (1972) A number of reports have appeared of the biosynthesis isolation and characterization of intermediate forms of mammalian proinsulins in various species (Kemmler et al 1973 Nolan et al. 1971 Tager et al 1973 Clark and Steiner 1969 Tung and Yip 1969 Chance 1971 Kitabchi et al 1972).



Fig 10
Hypothet al proinsuli hexamer arrangement as viewed along the three-fold axis of th h amer The onnecting peptide portion shown in lighter gray around th periphery of th darker outline of an in ulin hexamer arra ged according to th dat of Blundell et al (1971) Th ntral density repres nts th tw zinc atoms in oordination linkage to th (3 abov nd 3 below h xamer plane) hi tidin id chain t position 10 in the B hain

3 The biosynthetic organization of the beta cell

The beta cells of the islets of Langerhans share many features with other cells that elaborate secretory proteins (Fig. 11) The participation of the Golgi apparatus in the formation of beta granules was suggested as early s 1944 by Hard and coworkers (Hard, 1944) Later Munger (1958) confirmed by electron microscopy that secretion granule formation occurred within the Golgi apparatus. He identified progranules with altered morphology near the Golgi body Subsequent studies by electron microscopic radioautography (Howell et al. 1969 Orci et al 1971) have confirmed that newly synthesized peptide material passes in the Golgi apparatus into beta cell secretory granules. The overall process appears to be strikingly similar to that occurring in the pancreatic exocrine cells (Jamieson and Palade 1967 a b) and in many other secretory cells

It is now well established that proinsulin, in common with many other exportable proteins is synthesized by ribosomes associated with the rough endoplasmic reticulum (Permutt and Kipnis 1972) and it comprises the major biosynthetic product found in the microsomal fraction (Sorensen, et al. 1970) Although short pulse labeling experiments with intact islets have not indicated the synthesis

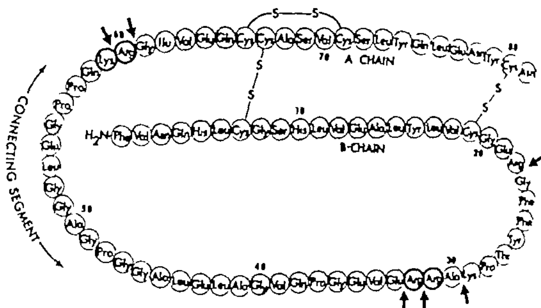


Fig. 9
Covalent structure of bovine proinsulin. Arrows indicate sites of tryptic cleavage. (Reproduced in modified form from Nolan et al. 1971)

either inactive or several orders of magnitude less active than their active derivatives.) The connecting peptide also does not obscure appreciably those monomer surfaces which interact in the formation of dimers and hexamers (Frank and Veros 1970; Steiner 1973). A hypothetical arrangement of the connecting peptide moiety in a proinsulin hexamer is shown in Fig. 10. This hexameric structure, having the C peptide oriented externally, may play a role in the efficient conversion of proinsulin to insulin in the beta cells. The three-dimensional structure of proinsulin has not yet been determined. Crystallization has been accomplished by Low and coworkers (Fullerton et al. 1970; Rosen et al. 1972; Low et al. 1974); however, further progress by means of X-ray analysis is eagerly anticipated.

2 The precursor relationship of proinsulin to insulin

By means of isolated islet preparations (Moskalweski 1965; Lacy and Kostianovsky 1967) the precursor-product relationship between proinsulin and insulin have been carefully documented in a variety of biosynthetic experiments (Steiner et al. 1967; Tung and Yip 1968; Lin and Haist 1969; Morris and Korner 1970; Tanese et al. 1970). The proteolytic conversion of proinsulin to insulin is a strictly intracellular process (Steiner et al. 1967). Conversion proceeds normally in the presence of cycloheximide, indicating that continuous protein synthesis is not necessary for the transformation of proinsulin to insulin (Steiner et al. 1967). By carefully characterizing rat islet proteins labeled with various precursor amino acids by mean of polyacrylamide gel electrophoresis, Clark and Steiner (1969) have shown the existence of two proinsulins, one corresponding to each of the two known rat insulins (Smith 1966). Both rats and mice (Markussen 1971 b) appear to have two non-allelic insulin genes, each coding for a proinsulin. The two rat proinsulins and their corresponding C-peptides have been isolated and their structures are known (Sundby and Markussen 1972; Markussen and Sundby 1973; Tager and Steiner 1972)

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Hypothetical proinsulin hexamer arrangement as viewed along the three-fold axis of the hexamer. The connecting peptide portion is shown in lighter gray around the periphery of the darker outline of an insulin hexamer arranged according to the data of Blundell et al. (1971). The central density represents the two zinc atoms in coordination linkage to the (3 above and 3 below hexamer plane) histidine side chains at position 10 in the B chain

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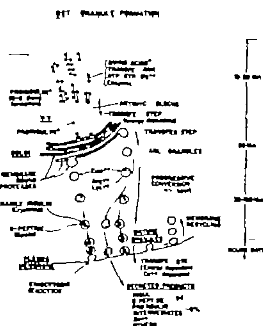


Fig 11
Schematic summary of the insulin biosynthetic machinery of the pancreatic beta cells See text for details regarding this process (R E R = rough endoplasmic reticulum M V = micro-vesicles) (Reproduced from Steiner and Rubenstein 1973)

of larger precursor forms than proinsulin recent studies in several laboratories suggest that a variety of secreted proteins are be synthesized initially with amino terminal extensions of 20-30 residues (Milstein et al 1972 Kemper et al 1974 Boime et al 1975) These extensions appear to serve as signal regions for the binding of the polyribosomes to the rough endoplasmic reticulum leading to the vectorial discharge of the newly formed peptides into the cisternae of the endoplasmic reticulum (Blobel and Sabatini 1971) These extensions normally are rapidly cleaved away in the microsomes but are retained when appropriate m RNA fractions are translated in various cell-free systems *in vitro* Similar results have recently been obtained when proinsulin m RNA from rat islets or whole fetal calf pancreas is translated in a cell free system derived from wheat germ (Lernmark et al. 1976 Lomedico and Saunders 1976 Chan et al 1976) The molecular weight of the bovine and rat preproinsulins is approximately 11,500-12 000 By means of automated Edman degradation the rat preprohormone has been shown to

Partial Amino Acid Sequence of Rat Preproinsulin

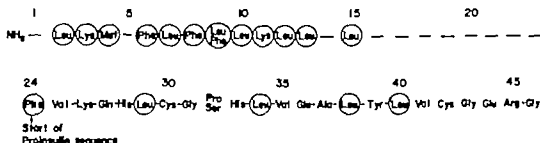


Fig 12
Partial amino acid sequence of rat preproinsulin Nucleic acid fractions containing the m RNA for proinsulin were extracted from isolated rat islets and translated in a cell-free system derived from wheat germ Rat preproinsulin was then isolated by immunoprecipitation and a partial amino acid sequence was determined by automated Edman degradation of the peptide labeled with various radioactive amino acids (for further details see Chain et al 1976) The residues identified by this procedure are shown enclosed within circles The known sequence of rat proinsulin begins at residue 24 as verified by the identification of phenylalanine at position 24 and leucines at positions 29 34 38 and 40

have 23 additional amino acids at the amino terminus as shown in Fig. 12 (Chain, et al 1976). As mentioned above, however, these preproinsulins appear to be converted to proinsulin so rapidly under normal conditions that they escape detection (Lernmark, et al 1976).

On the basis of the foregoing structural studies, certain predictions can be made regarding the structure of the gene(s) in the nuclear DNA that encode the preprohormone (Fig. 13). Recent advances in techniques for the isolation of genes (Brown and Stern, 1974) make it technically feasible to undertake the isolation of the gene(s) for proinsulin. To do this will first require the isolation of the proinsulin messenger RNA from of beta cells in pure form. This mRNA can then be used with reverse transcriptase enzymes to transcribe copies of portions of one of the DNA strands of the proinsulin gene for use as a probe in identifying this gene in the chromosomal DNA. The ultimate availability of "proinsulin-DNA" will also enable measurements to be made of the amount of proinsulin-mRNA per beta cell and it should then be possible to learn more about how insulin production is regulated by glucose, cAMP and other factors (Steiner, et al. 1972) at the levels of genetic transcription and of translation into protein.

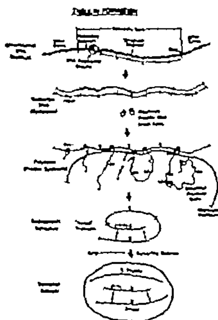


Fig. 13
Simplified scheme of the molecular biology of insulin formation. The proinsulin gene is represented schematically in the upper panel. RNA polymerase ... are for the transcription of proinsulin messenger RNA (m-RNA) from the gene and this then serves to guide the formation of proinsulin chains on the polyribosomes with the transient preproinsulin. (dashed lines indicate pre-sequence.)

There is no agreement as yet on the size of the islet polysomes actively engaged in the synthesis of proinsulin. One earlier study concluded that in rat islets these are mainly trisomes, a size consistent with a putative m-RNA length of about 258 nucleotides required to encode the 86 residue proinsulin polypeptide (Tjioe and Kroon, 1973). More recent reports suggest a somewhat larger translational unit size (Hew and Yip, 1974; Permutt and Kipnis, 1975), perhaps consistent with the larger m-RNA necessary to encode preproinsulin.

Caution must be exercised in attributing observed heterogeneity in circulating plasma components to normal biosynthetic precursors or intermediates. The tendency of insulin and proinsulin to form small amounts of covalently cross-linked dimers and higher aggregates (Nolan et al. 1971; Steiner et al. 1972) is now well established. The chemical nature of the cross linkages has not been elucidated but these aggregates are evidently formed in small amounts during isolation of insulin under acidic conditions and they possibly also can form during crystallization. Such aggregates occur at a low level in virtually all preparations of crystalline insulin unless special measures are taken to eliminate them (Nolan, et al. 1971; Schlichtkrull et

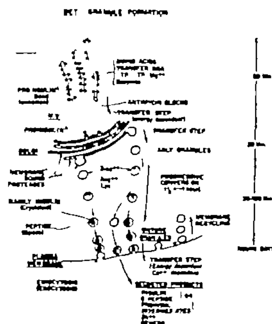


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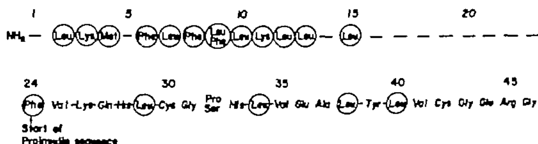


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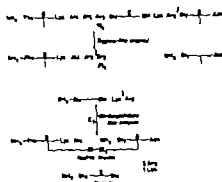


Fig. 14
Steps in the cleavage of proinsulin by the combined action of trypsin-like and carboxypeptidase B-like proteases. (See text for further discussion of this model system.)

to that of carboxypeptidase B. The latter enzyme is necessary to remove the C-terminal basic residues left after tryptic cleavage giving rise to the important naturally occurring products, the C-peptide and native insulin. We have shown that appropriate mixtures of pancreatic trypsin and carboxypeptidase B can quantitatively convert proinsulin to insulin *in vitro* (Kemmler et al. 1971). This model system can account for the known major intermediate forms and products that occur naturally in pancreatic extracts (Nolan, et al. 1971; Steiner, et al. 1971). In some species such as rat, additional cleavages occur in the C-peptide region of proinsulin which appear to be due to a protease having chymotrypsin-like activity (Tager, et al. 1973; Chance 1971). The role of this additional C-peptide cleavage in conversion remains unclear; however, and it probably occurs only in species where the proinsulin C-peptide contains sites of high chymotryptic sensitivity (Fig. 15). These findings suggest that the beta granules may contain a mixture of proteases, many of which are similar to those that occur in the exocrine pancreas. Thus the specific cleavage of precursor forms may be dictated partially by the high sensitivity of certain regions in the substrate molecules to a variety of known proteases, as well as by restricted specificities or special substrate adaptations on the part of the converting proteases.

As mentioned above, structural data on insulins and proinsulins in fishes suggest that the conversion of proinsulin to insulin in many of these species may be carried out by trypsin-like enzymes (Yamaji, et al. 1972; Steiner, et al. 1974). A trypsin-like protease has been detected in cod fish islet tissue by Grant and coworkers (Grant and Coombs 1971; Grant, et al. 1971). However, in the hagfish (a primitive cyclostome), the presence of a C-terminal neutral residue (Met) on the insulin B chain, as in the higher forms, suggests the additional requirement of a carboxypeptidase (Peterson, et al., 1974). Carboxypeptidase B activity is known to occur in the exocrine pancreas of several species of

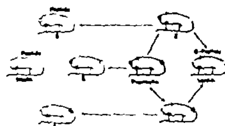


Fig. 15
Diagrammatic representation of the pathways for the conversion of proinsulin to insulin in the rat. Structures 1 to 5 represent possible proinsulin-like intermediates. The major pathway for proinsulin conversion is shown in heavy line. The sites on either side of the C-peptide filled by pairs of basic amino acid residues are indicated by circles and squares. Appropriate cleavage at these sites to yield insulin and C-peptide requires both trypsin-like and carboxypeptidase B-like activities. The slash represents a chymotrypsin-sensitive site in the C-peptide region of proinsulin. The NH_2 terminal of the A and B chains of insulin are indicated by dots. (Reproduced from Tager, et al. 1973.)

al 1972 Steiner et al 1972) These also have been detected previously in acid-ethanol extracts from an islet cell tumor (Melani et al 1970 b) In the latter case the higher molecular weight material contained both proinsulin and insulin immunoreactivity in a form that could not be converted to insulin like components by tryptic digestion. Insulin or proinsulin also may form complexes with abnormal plasma proteins in some patients as appears to be the case with big-big insulin (Yalow and Berson 1973 Sramkova et al 1975) Alternatively some tumors could conceivably secrete preproinsulin due to failure of cleavage of the preprohormone There is at present however no clear evidence of the existence of such biosynthetic abnormalities in beta cell tumors On theoretical grounds it seems unlikely that preprohormones would be secreted since the presequence and its cleavage appear to be necessary for the initial sequestration of the secretory product Failure to cleave the presequence might leave the proinsulin peptide embedded in the microsomal membrane

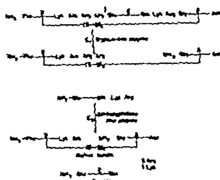
After biosynthesis peptide chain folding and sulfhydril oxidation have occurred in the microsomes proinsulin is transported to the Golgi apparatus (Fig 11) by a process that requires from 10 to 30 minutes (Howell et al 1969 Jamieson and Palade 1967 a & b Orci et al 1971 Steiner et al 1972) Addition to pancreatic islets of antimycin or other energy poisons after short labeling periods with ^{3}H leucine completely blocks the subsequent transformation of the newly formed proinsulin to insulin (Steiner et al 1970) However if the addition of antimycin is delayed until about 30 minutes after the beginning of the post labeling period there is no inhibition of subsequent conversion suggesting that once newly synthesized proinsulin has reached the Golgi apparatus its transformation no longer requires energy Howell using electron microscopic radioautography has demonstrted that dinitrophenol prevents the transfer of labeled proteins from the rough endoplasmic reticulum to the Golgi apparatus in the beta cells (Howell 197?) The chemical basis for this energy requirement in the intracellular translocation of peptides (Jamieson and Palade 1968) is not known

The conversion of proinsulin to insulin in intact rat islet cells behaves like a first order reaction having a half-time of about one hour (Steiner 1967) Peak labeling in the Golgi apparatus occurs at about 30 minutes after labeling the cells with relatively little radioactivity remaining in this region after an hour (Howell et al 1969 Orci et al 1971) Thus it is likely that conversion is initiated in the Golgi apparatus or in newly formed secretion granules or progranules as these leave the Golgi region, and that it continues for many hours within the granules as they collect in the cytosol (Fig. 11)

4 Mechanism of conversion of proinsulin to insulin

Our hypotheses regarding the mechanism of conversion of proinsulin to insulin are based on several lines of evidence including (1) the known structures of the cleavage products and of a number of intermediate forms (2) model studies with known proteolytic enzymes and (3) the detection of converting enzyme activities in whole islet preparations or appropriate subcellular fractions (Steiner et al 1974) To date these approaches have not provided definitive evidence on the origin specificity or subcellular localization of the converting proteases Several activities have been reported which produce insulin-like material from proinsulin in extracts of whole pancreas (Yip 1971) or in homogenates of islets of Langerhans (Zühlke et al 1974 Smith 197? b Smith and Van Frank 1974 Sorensen et al 197?) but the intracellular origin and mechanism of action of these enzymes remain uncertain

The major types of proteolytic cleavage required for the conversion of proinsulin to insulin are shown in Fig 14 This scheme envisions the combination of a trypsin like protease with another having specificity similar



F g 14
Stage in the clea ge of proinsulin by the ombined action of trypsin-like and arboxy peptidas B-like proteases (See t xt for furt her discussion of this model sy tem)

to that of carboxypeptidase B The latter enzyme is necessary to remove the C-terminal basic residues left after tryptic cleavage giving rise to the important naturally occurring product the C-peptide and native insulin W have shown that ppropriate mixtures of pancreatic trypsin and carboxypeptidase B can quantitatively convert proinsulin to insulin *itro* (Kemmler et al 1971). This model ystem can account for the known major intermediate forms and product that occur naturally in pancreatic extracts (Nolan, et al. 1971 Steiner et al 1973) In some species, such rat additional cleavages occur in the C-peptide egion of proinsulin which appear to be due to protease having chymotrypsin-like activity (Tager et al. 1973 Chance 1971) The role of this additional C-peptide cleavage in conversion remains unclear however and it probably occurs only in pe cies where the proinsulin C-peptide contains sites of high chymotryptic sensitivity (Fig. 15) These findings suggest that the beta granules may contain a mixture of proteases, many of which are milar to those that occur in the exocrine pancreas Thus the specific lea age of precursor forms may be dictated partially by the high sensiti ity of certain regions in the substrate molecules to a variety of known proteases, as well as by restricted specificities or special substrate adaptations on the part of the converting proteases.

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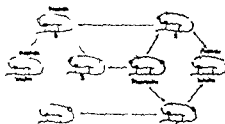


Fig 15
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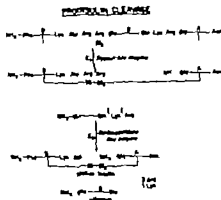


Fig 14
Stage in the cleavage of proinsulin by the combined action of trypsin-like and carboxypeptidase B-like proteases (See text for further discussion of this model system)

to that of carboxypeptidase B The latter enzyme is necessary to remove the C-terminal basic residues left after tryptic cleavage giving rise to the important naturally occurring products, the C-peptide and native insulin. We have shown that appropriate mixtures of pancreatic trypsin and carboxypeptidase B can quantitatively convert proinsulin to insulin *in vitro* (Kemmler et al. 1971) This model system can account for the known major intermediate forms and products that occur naturally in pancreatic extracts (Nolan, et al. 1971 Steiner et al. 1971)). In some species, such as rats, additional cleavages occur in the C-peptide region of proinsulin which appear to be due to a protease having chymotrypsin-like activity (Tager et al. 1973 Chance 1971) The role of this additional C-peptide cleavage in conversion remains unclear however and it probably occurs only in species where the proinsulin C-peptide contains sites of high chymotryptic sensitivity (Fig. 15) These findings suggest that the beta granules may contain a mixture of proteases many of which are similar to those that occur in the exocrine pancreas. Thus the specific cleavage of precursor forms may be dictated partially by the high sensitivity of certain regions in the substrate molecules to a variety of known proteases as well as by restricted specificities or special substrate adaptations on the part of the converting proteases.

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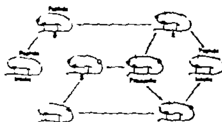


Fig 15
Diagrammatic representation of the pathways for the conversion of proinsulin to insulin in the rat Structures 1 to 5 represent possible proinsulin-like intermediates The major pathway for proinsulin conversion is shown in heavy lines The sites on either side of the C-peptide filled by pairs of basic amino acid residues are indicated by circles and squares Appropriate cleavage at these sites to yield insulin and C-peptide requires both trypsin-like and carboxypeptidase B-like activities The slash represents a chymotrypsin-sensitive site in the C-peptide region of proinsulin. The NH terminal of the A and B chains of insulin are indicated by dots (Reproduced from Tager et al 1973)

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More recently we have detected low levels of [^{3}H]TAME esterase activity in islet homogenates and have also observed that small amounts of esterase activity are liberated from intact islets during incubation *in vitro* (Steiner et al 1975) The amount of enzymatic activity released is increased by glucose combined with the phosphodiesterase inhibitor IBMX (3-isobutyl-1 methyl xanthine) both stimulators of insulin secretion This approach may provide a convenient means for further characterizing these proteases under conditions limiting the presence of contaminating cellular proteases Of considerable interest is the *possible relationship* of this activity to the *trypsin-like plasminogen activator that is known through the work* of Reich and coworkers to be produced by a *wide variety of cells in vivo* or *in culture* (Unkeless et al 1974)

It has been less difficult to demonstrate the presence of carboxypeptidase B-like activity in crude granule lysates using ^{3}H-arginine labeled peptides derived from ^{3}H-arginine labeled proinsulin by treatment with limiting amounts of trypsin i e insulin (B chain) Arg-Arg-COOH and C peptide Lys-Arg COOH (Kemmler et al 1973) When these substrates were incubated for 5 hours with granule lysates prepared from rat islets the lysates liberated approximately 80% as much free labeled arginine as did treatment with excess carboxypeptidase B These results indicate the presence of an exopeptidase *in the granule fraction that* has cleavage specificity similar to that of carboxypeptidase B

To study this activity in greater detail we *have recently utilized a fluorometric proce*dure using hippuryl arginine as the substrate and *fluorescamine to detect the release of* free arginine With this method it is possible to detect carboxypeptidase *B like activity* (inhibited by EDTA and *o* phenanthroline but not by DFP or *iodoacetamide) in homo*genates of whole islets as well as in the incubation medium of incubated islets (*Zühlke* and Steiner 1975) These results thus confirm our earlier findings of exopeptidase activity directed toward C-terminal basic residues in lysed granule fractions (*Kemmler et al* 1973) and provide further evidence that a carboxypeptidase *B-like enzyme is present in* the beta cells

Zühlke and coworkers (Zühlke et al. 1974) have recently shown that disrupted secretion granule fractions convert proinsulin to insulin-like components in a DFP-sensitive reaction when incubated at pH 6.5 with considerably higher concentrations of proinsulin than were used in our earlier experiments (Kemmler et al. 1973). These workers also ha e described the splitting of a synthetic heptapeptide derived from the amino acid sequence of porcine proinsulin (i.e. residues 28-34 having ^{14}C-arginine at position 31) by islet homogenates and crude secretion granule fractions (Zühlke, Jahr Schmidt and Kirschke unpublished. Free ^{14}C-arginine could be detected providing evidence for the action of both an endopeptidase and a carboxypeptidase B-like enzyme.

Son, Lin and Hurst (1973) have provided evidence that the proinsulin converting activity of disrupted granule fractions is localized in a membrane-containing fraction Unfortunately this fraction was not characterized. Such a membrane association of the endopeptidase activity might limit proteolytic activity *in vivo* and thus afford means for protecting insulin from further proteolytic degradation in the granules. The possibil ty that the converting enzymes are related to some of the lysosomal proteases has been considered by several workers (*Steiner et al 197*? Smith, 1972 b) However little of the *currently available* information on these enzymes is consistent with lysosomal origin (for a more extensive discussion, see Steiner et al 1972).

The elationship of the proinsulin-transforming enzymes to the exocrine pancreatic proteases poses interesting questions Some investigators believe that the islet cells and the exocrine pancreatic cells share a common embryological derivation from the endoderm of the gut (Wessells and Evans 1968 Pictet et al 1973) Moreover Rutter and coworkers have shown that trypsin and carboxypeptidase B appear together at a later time during the embryonic development of the pancreas than do many of the other pancreatic hydrolases (Pictet and Rutter 1972). This behaviour suggests that these two enzymes are closely coordinated both developmentally as well as functionally

The recent finding of Frazier Angeletti, and Bradshaw (1972) of sequence homologies between proinsulin and the mouse submaxillary gland nerve growth factor also would seem consistent with an endodermal origin of the beta cells Further comparative structural studies of proinsulins from primitive vertebrates will hopefully shed more light on its evolutionary origin as well as on the origin and evolution of its associated cleavage enzymes (Steiner et al. 1973)

5 The formation of beta granules

Morphological studies of newly formed secretory granules in a variety of cells suggest that these particles undergo biochemical maturation after their formation in the Golgi apparatus (see Fig 11) Thus in the beta cells the progranules characteristically are less dense than the mature granules inclusions and have a uniform density throughout (Munger 1958). A variety of biochemical changes may take place in these granules as they remain in the cytoplasm of the cell including the proteolysis of proinsulin to insulin. Morphological studies of mature insulin secretion granules indicate that the dense central inclusion may consist mainly of crystalline insulin packed with repeat-unit spacings that are closely similar to those observed in ordinary zinc insulin crystals (Greider et al. 1969 Lange, et al. 1972) Thus, as insulin is liberated from proinsulin it evidently tends to crystallize with zinc. The C-peptide liberated in the conversion process probably remains in the clear space surrounding the dense insulin crystal since there is no evidence for co-crystallization of the C-peptide with insulin.

The role of zinc in secretion granule formation is not understood. The available evidence indicates that most of the islet zinc is present in the granules and is liberated propor

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These peptides exhibit a much higher rate of mutation acceptance than do the corresponding insulins finding consistent with the possibility that this region in the proinsulin molecule does not contain an active center for a specific hormonal function. Among known proteins, only the fibrinopeptides have a higher rate of mutation acceptance than the proinsulin C-peptides The much lower rate of mutation acceptance of insulin however is similar to that of many other functional proteins such as hemoglobin or cytochrome (Dayhoff 1972). Certain regions of the relatively large connecting peptide may serve specific functions such as facilitating the folding of the proinsulin polypeptide chain and the formation of the correct disulfide bonds (Steiner and Clark, 1968) or guiding the enzymatic cleavage of proinsulin to insuli Several acidic residues are consistently present in the connecting peptides These tend to offset the cationic charges due t the basic residues at the cleavage sites so that the isoelectric pH of proinsulin is nearly the same as that of insulin, e pH 5 1 5 5 (Steiner et al 1972 Kohnert, et al 1972 Kohnert, et al 1973). It is also possible that translational constrai ts such as mRNA sequence size or secondary structure may play a role in dictating the primary structure of some regions within proinsulin.

Synthesis of several mammalian C-peptides has been accomplished recently by classical fragment condensation approaches (Geiger et al 1969 a Yanaihara, et al 1970 Yanaihara et al 1972 a, Yanaihara, et al. 1972 b Naithani 1977 Naithani 1973 a and b). The synthetic porcine C-peptide containing all four terminal basic residues was tested for i ability to promote the recombination of insuli A and B chains *in vitro* but it failed to influence the yield (Geiger et al 1969 b). Synthetic porcine and bovine C-peptides cross-react well with antibodies directed against the corresponding natural proinsulins or C-peptides and fragments of these peptides have been successfully utilized to study the antigenic determinants in this region of the proinsulin molecule (Yanaihara et al. 1972 a Yanaihara, et al 1972 b Naithani 1973 a & b).

tionately with insulin *during secretion* (Falkmer 1971 Logothetopoulos et al 1964) However the mechanism for accumulation of zinc within the granules is not known. Both proinsulin and insulin have been shown to bind zinc (Frank and Veros 1970 Grant, et al 1972) However the insulins of a few species including the guinea pig and coypu (Smith 1966) and the hagfish (Peterson et al 1974 Steiner et al 1973) lack the histidine residue at position 10 of the B chain required for zinc binding during the association of insulin dimers into hexamers (Blundell et al 1971) As mentioned earlier most mammalian proinsulins probably also can exist as hexamers stabilized by 2 zinc atoms coordinated with the 6 B-10-histidines as in the case of insulin but these also evidently can bind larger amounts of zinc at additional sites without precipitating from solution (Grant, et al 1972) This intriguing finding suggests that proinsulin may play a role in zinc accumulation in the islet cells These metal ions might influence the conversion process in some way and then subsequently aid in the sequestration of the newly formed insulin in an osmotically inactive and biochemically stable crystalline form (see Fig. 11)

The pH of the granule interior is not known but we may assume that it is near pH 6 0 in the mature granules since the optimal pH for insulin crystallization *in vitro* also is about 6 0 (Kemmler et al 1973) However this pH is unfavorable for disulfide exhange reactions which occur more readily under mildly alkaline conditions Accordinly the pH in the cisternal spaces of the rough endoplasmic reticulum, where proinsulin folding and sulfhydryl oxidation occur may be somewhat above neutrality As the secretory products move to the Golgi apparatus and proteolysis begins the cationic arginine and lysine residues liberated during conversion may diffuse out of the granules and be replaced by hydrogen ions resulting in a decrease in the intragranular pH This gradual acidification as the granules mature could create appropriate conditions for the formation of the crystalline zinc insulin inclusions (Steiner and Rubenstein 1973) From the foregoing considerations it can be appreciated how closely and elegantly the biosynthesis of proinsulin its intracellular transport proteolysis and storage as insulin are integrated both topographically as well as biochemically within the beta cell

6 The C-peptide as a product of proinsulin transformation

Due to the localization of the conversion process within secretion granules, the C peptide accumulates along with insulin in equimolar amounts (Steiner et al 1971) and is secreted along with the hormone by exocytosis of the granule contents (Rubenstein et al 1969 a) The amino acid sequences of C-peptides from nine mammalian and one avian species have been combined in the composite diagram shown in Fig 16 (Dayhoff 1973 Ko et al. 1971 Markussen and Sundby 1972 Smith and van Frank, 1974 Oyer et al 1971 Peterson et al 1972 Tager and Steiner 1972).

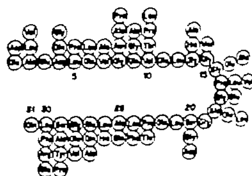


Fig 16
Amino acid sequence of human proinsulin C-peptide combined with the known substitutions occurring in 8 other mammalian and one avian C peptide shown alongside Deletions occur in the dog (residues 4 11) pig (residues 18 and 19) sheep and ox (residues 22 26) and guinea pig (residues 25 and 26) (These sequences do not include the basic residues at either end which link the C peptide to the insulin chains)

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Islet secretory products – proinsulin

1 Measurement of proinsulin in serum

A number of studies of normal human plasma or urine samples have indicated the presence of small amounts of immunoreactive material similar in molecular weight to proinsulin (Rubenstein et al. 1968; Roth et al. 1968). The structure of human proinsulin is shown in Fig. 17. Although it would be advantageous to measure human proinsulin and its intermediate fractions (proinsulin-like components, PLC) by direct immunoassay in unextracted serum, this has not been possible. The reasons lie in the cross-reactivity of proinsulin with insulin, on the one hand, and the C-peptide on the other. As all 3 of these peptides have been identified in the circulation, a preliminary step is required to separate them from each other. The most commonly used approach has involved gel filtration of serum followed by measurement of the column fractions in the insulin immunoassay. In our initial studies we extracted serum insulin and proinsulin into acid ethanol and separated the peptides by gel filtration on a Bio-Gel P 30 column equilibrated in 3 M acetic acid (Melani et al. 1970 a). The initial reason for choosing this technique was the reluctance to gel filter serum in neutral or alkaline buffers in which polymerization or aggregation of insulin might occur. In fact, this does not appear to be a problem. Another advantage is the ability to extract large volumes of serum and yet separate the hormones on relatively small columns. Furthermore it is easier to characterize the separated proinsulin and insulin under these conditions when most of the

HUMAN PROINSULIN

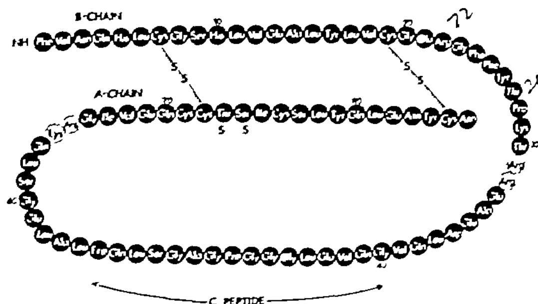


Fig. 17
Covalent structure of human proinsulin. The dashed circles indicate the pairs of basic residues removed in the conversion of the proinsulin to insulin in the beta cells. (Reproduced from Oyer et al. 1971)

other serum proteins have been removed. The most obvious disadvantage of the method is the length of time required for the procedure and the limitation on the number of samples that can be analyzed by one laboratory

Roth et al. (1968) have separated proinsulin and insulin on 1 × 50 cm columns of Sephadex G-50 fine equilibrated in a veronal buffer containing human serum albumin, rabbit fraction II and toluene One or two ml of serum is applied directly to the column, fraction sizes of 1 0 to 1.5 ml are collected and 0 4 to 0.8 ml aliquots taken for immunoassay We have modified this method to use a column of Bio-Gel P 30, equilibrated in the borate buffer which we use in the immunoassay Fractions can be collected directly into the immunoassay tubes, thus obviating the need for further pipetting at this stage. The void volume is determined by the elution position of ^{125}I-albumin or blue dextran 2000 while the salt peak is marked by Na ^{125}I The column is calibrated with tracers of ^{125}I-proinsulin and ^{125}I-insulin Because certain preparations of these labeled hormones may not elute identically with the native proteins it may be preferable to determine the characteristics of the column by assaying the elution positions of unlabeled insulin (2 ng) and proinsulin (2 ng).

When serum is directly applied to these columns, recoveries have been essentially complete. In order to calculate the absolute level of proinsulin and insulin, fractions of the earlier eluting peak are read from a human proinsulin standard, while those comprising the second peak are measured against a human insulin standard. As the supply of human proinsulin is limited at present, many investigators have expressed the values of proinsulin in terms of the insulin standard (Fig. 18)

Another method for separating insulin from proinsulin has been described by Kitabchi and his coworkers (Kitabchi et al 1971 Duckworth et al. 1972). These investigators

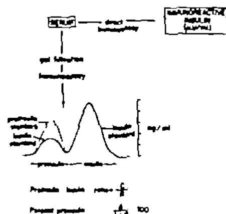


Fig 18
A schematic diagram illustrating th measurement of proinsulin and insulin in serum Serum is meas ed directly in the immunoassay (immunoreactive insulin – IRI) It is then gel filtered to eparate the proinsulin and insulin and each f action is assayed in the insulin radioimmunoassay Th early eluting peak (proinsulin-like-component) is ead from the human proinsulin standard curve (curve C) or alternatively from the human insulin tandard (curve A) The sum of the individual fractions in each peak (corrected for volume) is the proinsulin and insulin concentration spectively The elationship between proinsulin and insulin has been expressed in two ways the proinsulin to insulin ratio with each read from its appropriate standard and the percentage proinsulin where both peptides have been ead from the insulin standard (Reproduc d from. J.J Starr A.H Rubenstein Methods of Hormone Radioimmunoassay 1974 Academic Press Inc p 289)

have used an enzyme which is relatively specific for the proteolytic degradation of insulin, but not proinsulin. Measuring samples in an insulin assay before and after incubation with this enzyme (insulin specific protease ISP) should enable one to determine the relative concentrations of the two peptides. The accuracy of this method is limited, however especially at low serum immunoreactive insulin (IRI) concentrations, because the degra-

Islet secretory products – proinsulin

1 Measurement of proinsulin in serum

A number of studies of normal human plasma or urine samples have indicated the presence of small amounts of immunoreactive material similar in molecular weight to proinsulin (Rubenstein et al 1968 Roth et al 1968) The structure of human proinsulin is shown in Fig. 17 Although it would be advantageous to measure human proinsulin and its intermediate fractions (proinsulin-like components PLC) by direct immunoassay in unextracted serum this has not been possible The reasons lie in the cross-reactivity of proinsulin with insulin on the one hand, and the C-peptide on the other As all 3 of these peptides have been identified in the circulation a preliminary step is required to separate them from each other The most commonly used approach has involved gel filtration of serum followed by measurement of the column fractions in the insulin immunoassay In our initial studies we extracted serum insulin and proinsulin into acid ethanol and separated the 2 peptides by gel filtration on a Bio-Gel P 30 column equilibrated in 3 M acetic acid (Melani et al 1970 a) The initial reason for choosing this technique was the reluctance to gel filter serum in neutral or alkaline buffers in which polymerization or aggregation of insulin might occur In fact this does not appear to be a problem Another advantage is the ability to extract large volumes of serum and yet separate the hormones on relatively small columns. Furthermore it is easier to characterize the separated proinsulin and insulin under these conditions when most of the

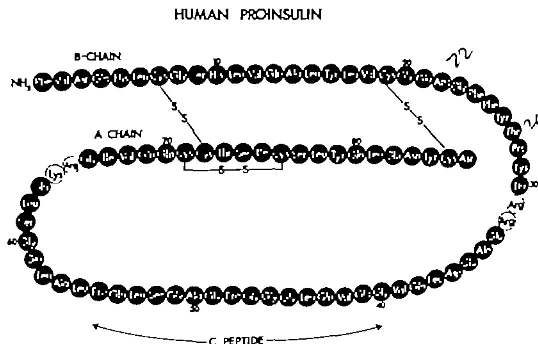


Fig 17
Covalent structure of human proinsulin The dashed circles indicate the pairs of basic residues removed in the conversion of the proinsulin to insulin in the beta cells (Reproduced from Oyer et al 1971)

of these components is still uncertain and further work is needed to characterize them fully (Fig. 19).

Nunes-Correa, et al. (1974) studied a patient who suffered from severe hypoglycemic attacks presumably on the basis of an islet cell adenoma. In addition to insulin and proinsulin they found a third peak in the patient's serum with molecular weight of 24,000. The material in this peak reacted with insulin antibodies and had approximately 50 to 100 % of the biological activity of insulin. It seems unlikely that this component could be related to either preproinsulin or proinsulin since neither form could be expected to have such a high level of biological activity.

3 Circulating proinsulin

The mean fasting proinsulin levels in normal subjects is 0.16 ± 0.2 ng/ml (Mako et al. 1973). In studies using a human insulin standard for measurement of both proinsulin and insulin, PLC comprises 15 % of the total immunoreactive insulin concentration (range 0-22 %). After oral glucose the levels of proinsulin rise slowly and peak later than insulin. When expressed as a percentage of the insulin concentration a decline from the fasting value is observed during the first 15 to 60 minutes (Gorden and Roth 1969). Thereafter proinsulin contributes an increasing amount to the immunoreactive insulin level. Obese patients with hyperinsulinemia have fasting concentrations of proinsulin and a greater absolute increase after glucose than normal weight subjects (Melani et al. 1970). However the high levels of proinsulin coexist with raised insulin concentrations, so that the relative proportions of the two polypeptides are generally in the same range observed in healthy subjects (Gorden and Roth, 1969).

Great interest has been expressed in the possibility that an altered proinsulin insulin ratio might occur in patients with diabetes mellitus. Initial results in a limited number of both normal weight and obese patients with mild

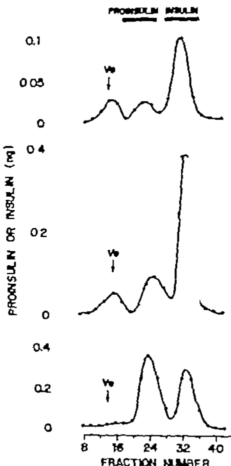


Fig. 19
Gel filtration patterns of sera from three patients with islet cell tumors. The samples in the upper and middle panels show three peaks which react in the insulin immunoassay. The second peak represents proinsulin-like-components and the third insulin. The nature of the large molecular weight early eluting peak is uncertain (See text). The lower panel shown for contrast demonstrates the absence of this material in another patient. (Reproduced from Rubenstein et al. 1973).

dation of insulin is generally incomplete This may be due in part to the presence of noncompetitive inhibitors of the enzyme in plasma (Cresto et al 1974 Starr et al 1975)

2. Characterization of circulating proinsulin-like components

In most studies PLC has been measured after gel filtration of sera on Sephadex or Bio-Gel columns equilibrated in acetic acid borate or veronal buffers Because of its higher molecular weight, PLC elutes before insulin and may be identified in either the insulin or human C peptide immunoassays However these methods do not differentiate the 2 chain proinsulin intermediates (Steiner et al 1968) from the single chain precursor and additional techniques are required to demonstrate their presence in the circulation.

Lazarus et al (1972) have described the presence of a proinsulin intermediate in addition to proinsulin in the serum of a patient with a surgically-documented carcinoma of the pancreatic islets The fasting total IRI concentration was approximately 1000 μU/ml and the percentage PLC was 85 % An acid alcohol extract of serum was electrophoresed on polyacrylamide gel and 3 peaks were identified. Two of these had mobilities corresponding to insulin and proinsulin while the third peak ran in an intermediate position The authors suggested that the migration behavior of this peak was compatible with desdipeptide proinsulin (Chance 1971) Although the biological activity of the PLC was almost 50 % that of insulin it is difficult to be certain of this result because of problems in standardizing the proinsulin components

In addition to this intermediate form other components which react in the insulin assay have been identified in sera of patients with islet cell tumors Thus Gorden, et al (1971 a) have described a proinsulin-like component which eluted ahead of the proinsulin marker on a 1.5 × 90 cm column of Sephadex G 50 in a subject with an islet cell carcinoma The PLC isolated from this patient s serum did not cross-react identically with a porcine insulin standard, whereas dilutions of plasma PLC from healthy subjects and other patients with tumors were indistinguishable from the porcine standard in this assay system. Its biological activity was 3 times greater than porcine proinsulin and it was converted to insulin by exposure to trypsin. It is of interest that the serum insulin component also did not exhibit immunological identity with a porcine insulin standard Because of the small amounts of material available further characterization was not achieved

Yet another form of immunoreactive insulin which has been named "big, big insulin" has been described in the plasma of an insulinoma suspect by Yalow and Berson (1973) This component had a molecular weight of about 180 000 was immunochemically identical to human insulin, was more basic than porcine or human insulin and was rapidly transformed by trypsin to an insulin like component Skrabkova et al (1975) have recently reported that this component yielded ordinary insulin when extracted with acid-ethanol Excess serum binding of insulin in this patient was apparently due to the production of an abnormal insulin-binding globulin by a plasmacytoma

The presence of a high molecular weight immunoreactive component in an acid ethanol extract of an insulinoma was described by Melani et al (1970 b) This material which eluted in the void volume of the Bio-Gel P 30 column comprised 10 % of the total immunoreactive insulin-like material It reacted in the C-peptide assay but was not converted to insulin or proinsulin by trypsin. In additional studies on insulinoma patients we have noted variable amounts of material eluting in the void volume of Bio-Gel P 30 columns which react with insulin antibodies Berson and Yalow (1973) also have found small amounts (0 7 1 0 %) of such components in acid-ethanol extracts of 2 islet cell adenomas and a normal pancreas The origin and significance

ram PLC estimations may be useful in diffe rentiating benign beta cell tumors from carci nomas. The basal PLC percentage was 58 and 70 in 2 of our patients with malignant tumors but values in this range (above 50 %) were also noted in 5 subjects with adenomas. Simi lar results were found by Sherman et al. (1972) (2 patients with malignant tumors had percentage PLC of 62 and 76 while 5 adenoma cases also had values higher than 50 %). The patient with a malignant tumor had the highest percentage PLC (89 %) in the study of Gutman et al (1971) while Gorden et al. (1971 b) reported values of 38, 46 53 and 78 % in 4 such patients Although Blackard et al (1970) did not measure basal PLC the percentage 2 hours after oral glucose was 53 % The finding of Pearson, et al. (1972) is also of interest in this regard because the percentage PLC in their patient with an islet cell carcinoma rose to 80 % over an 8-week period. The authors concluded that the per centage PLC may rise as loss of tumor differentiation occurs. Nevertheless, as many drugs and continuous glucose infusions were administered during this time the results should be interpreted with caution. Very high PLC values in a further 2 patients with carci nomas have also been reported by Taylor et al (1970) and Lazarus, et al (1970 b)

These results suggest that most patients with malignant islet cell tumors do have a markedly elevated percentage PLC However a sig nificant number of subjects with adenomas also fall into this high range On the other hand it seems that the finding of low percentage PLC in patient with an islet cell tumor does favor the diagnosis of a benign lesion.

4 Peripheral metabolism of proinsulin

Proinsulin comprises 2 to 9 % of the immunoreactive insulin-like material in normal pancreas (Rastogi, et al 1970 Sando et al. 1972 a, Sando and Grodsky 1973). This value is similar to that found in the portal vein of man (Horwitz, et al. 1975 c) but much lower than in peripheral serum. This discrepancy can be explained by the slower metabolism of proinsulin compared to insulin. Thus the immunological half-life of intravenously injected porcine proinsulin was 18 to 20 minutes and that of insulin 8 and 6 minutes in baboons and swine, respectively (Stoll et al 1970) Similar values were found after injection of bovine proinsulin and insulin in dogs (Rubenstein et al 1970 a Sonksen, et al 1973) infused porcine insulin and proinsulin into healthy subjects and showed a mean metabolic clearance rate of 13.3 ml/kg/min for insulin and 3 1 ml/kg/min for proinsulin with mean half-lives of 4 4 and 25.6 minutes respectively The half disappearance time of endogenous proinsulin (18-25 minutes) was markedly slower than that of insulin (3-4 minutes) in three patients following removal of their islet cell tumors (Starr and Rubenstein 1974) It should also be noted that there is no evidence for conversion of proinsulin to insulin (Fig. 21) in the circulation (Rubenstein, et al. 1969 b).

In studies in rats the metabolic clearance rate (MCR) of bovine insulin (16 4 ± 0.4 ml/min) was significantly greater than that of proinsulin (6 7 ± 0.3 ml/min) The MCR of both polypeptides was independent of plasma levels over a wide range of steady state plasma concentrations varying from 1 15 ng/ml In contrast to the differences in their MCR the renal disposition of the two polypeptides was similar being characterized by high extraction and very low urinary clearance (Katz and Rubenstein 1973) The renal arteriovenous differences of proinsulin and insulin averages 36 and 40 % respectively and was linearly related to their arterial concentration between 2 and 25 ng/ml. The fractional urinary clearance never exceeded 0 6 % indicating that more than 99 % of the amount filtered was sequestered in the kidney

On the other hand studies on the removal of bovine proinsulin and insulin by the isolated perfused rat liver have shown that the hepatic extraction of proinsulin is considerably slower than that of insulin at both high and low

diabetes characterized only by glucose intolerance demonstrated basal and post-glucose responses indistinguishable from control subjects (Melani et al 1970 a Gorden and Roth 1969 Gorden et al 1971 b) More recently Duckworth et al (1972) using the insulin specific protease to measure insulin reported that both obesity and carbohydrate intolerance were associated with slightly increased *PLC levels* but that the coexistence of the 2 conditions especially in older diabetics was marked by significantly elevated *PLC concentrations and a rise in the PLC IRI* ratio In contrast children with mild diabetes and elevated immunoreactive insulin levels have proinsulin values within the normal range in both the fasting and stimulated state (Rosenbloom et al 1975)

There are a number of conditions which are characterized by an elevated proinsulin insulin ratio Gorden et al (1972) showed that in 6 patients with severe hypokalemia of diverse etiologies the PLC formed a greater proportion of the total IRI in the basal and post stimulated state These subjects were glucose intolerant exhibited delayed and low insulin responses and the high PLC percentage was at least partly a result of their insulinopenic state Correction of the hypokalemia reversed this abnormality In severe diabetics and other patients with low fasting insulin levels a similar situation may be found (Gorden et al 1974) Mako et al. (1973) have pointed out that the absolute concentration and percentage PLC in the basal state in patients with chronic renal failure are markedly elevated and that the values may overlap those found in subjects with islet cell tumors The reason for this finding lies in the critical role of the kidney as the major organ involved in proinsulin degradation (Katz and Rubenstein 1973)

The major clinical significance of a raised serum proinsulin concentration has been in *the diagnosis of pancreatic* islet cell tumors. We have studied 17 patients with beta cell adenomas and 2 with carcinomas (Rubenstein et al 1974) The absolute basal PLC concentrations varied between 0 23 and 17 48 ng/ml Only three patients overlapped the normal range (038-0 45 ng/ml) The percentage PLC ranged from 2 9 to 7 (normal values 4 6 to 22 8 %) and only 4 of the insulinoma group fell within the range of the control subjects (Fig 20) These results are similar to those of Sherman et al (1972) who described three of 21 islet cell tumor patients with basal IRI concentrations within their normal range, but only one with a normal proinsulin concentration Four subjects had percentage PLC which overlapped their controls The findings in these two studies are representative of the conclusions in a number of other reports (Blackard et al 1970 Goldsmith et al 1969 Pearson et al 1972 Lazarus, et al 1970 b Gorden et al 1971 b Gutman, et al 1971)

We have considered the possibility that se-

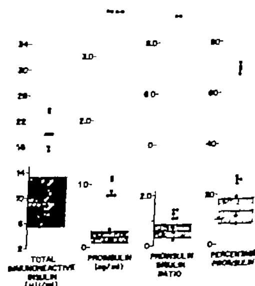


Fig 20
Basal total immunoreactive insulin (IRI) proinsulin-like-components proinsulin insulin atio and percentage proinsulin in 19 patients with islet cell tumors The mean ± 1 S.D of 46 control subjects is shown in the hatched area (Reproduced from Rubenstein et al 1973)

not this particular enzyme (ISP) is important in the degradation of insulin under physiological conditions is still uncertain and further experiments to elucidate the mechanism and localization of insulin degradation in the liver and kidney are necessary

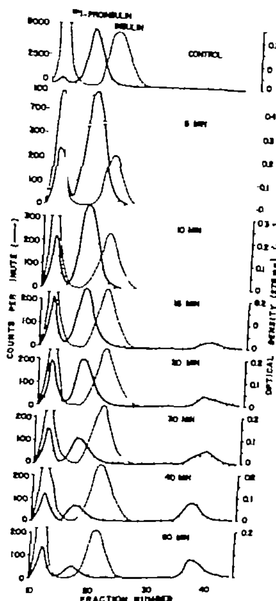


Fig 21
Gel filtration of plasma samples at 5-60 minutes after intravenous injection of ^{131}I proinsulin into normal rats 0 1 ml plasma and 1 mg bovine insulin were mixed and then separated on a 1 × 50 cm column of Sephadex G – 50 equilibrated in 1 M acetic acid An increase in degradation products (fractions 36-40) is seen with time (Reproduced from Rubenstein et al 1969)

concentrations (Fig. 22) That this finding was probably not related to the heterologous system used was demonstrated by comparing

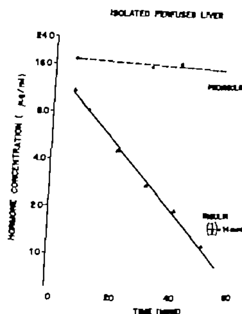


Fig 22
Removal of insulin and proinsulin by the isolated perfuse rat liver The insulin was rapidly removed with a t 1/2 of 14 mins while the removal of proinsulin was very slow (Reproduced from Rubenstein et al 1972)

the degradation of labeled rat and bovine proinsulin by both rat and bovine liver homogenates (Rubenstein et al 1972 c) Stoll et al. (1970) have also shown using an isolated perfused liver that porcine monocomponent insulin was cleared with a T 1/2 of 17 minutes while there was no significant clearance of porcine proinsulin.

A protease which is relatively specific for insulin and does not significantly degrade proinsulin has been described by Brush (1971) who isolated the enzyme from muscle and by Burghen et al (1972) who found a similar enzyme in liver Studies by Kitabchi and Stentz (1972) on the degradation of insulin and proinsulin by rat organ homogenates showed variation in the relative ability of different tissues to degrade insulin and proinsulin Only pancreas and kidney homogenates degraded immunoreactive proinsulin at a rate greater than 10 % that of insulin. Whether or

not this particular enzyme (ISP) is important in the degradation of insulin under physiological conditions is still uncertain and further experiments to elucidate the mechanism and localization of insulin degradation in the liver and kidney are necessary

Islet secretory products – C-peptide

Because insulin and the C-peptide are derived from one molecule of proinsulin and are stored and released from the pancreas essentialy in equimolar quantities (see Section 4) the serum concentrations of these peptides should correlate well This supposition has recently been confirmed (Block et al 1972 a Horwitz et al 1973) and certain clinical situations in which the determination of serum C peptide levels is particularly useful as a measure of beta cell function have been recognized

1 Measurement

Widespread availability of the human C-peptide immunoassay has been hindered by several factors First because of the poor cross-reactivity between human C-peptide and all the other C peptides which have been studied (Rubenstein et al 1970 b) it has been appreciated that human C-peptide must be used for standard radioactive label and as antigen for the production of antibodies Second human C-peptide has a relatively low molecular weight (3021 daltons) and thus tends to be a poor antigen Even when coupled to large proteins such as bovine serum albumen immunization with C-peptide does not consistently result in a high-titer antiserum in either rabbits or guinea pigs It is possible that a lack of rigid secondary or tertiary structure (Frank and Veros 1968 Markussen 1971 b) renders this peptide poorly immunogenic

A human C-peptide radioimmunoassay was initially developed in this laboratory using C-peptide extracted from human pancreases removed at autopsy (Melani et al 1970 c) Recently however antisera have been produced more readily from synthetic C-peptide (Yanaihara, et al 1970) In order to iodinate the C-peptide to high specific activity a tyrosyl residue must be added because there is no tyrosine or histidine residue in any of the known natural peptides (Melani et al. 1970 c) Tyrosyllated C-peptides have also been prepared synthetically by Yanaihara and his coworkers Because of minor differences in immunoreactivity between endogenous and synthetic human C-peptide, we have continued to use the endogenous peptide for our standard. The method for extracting and purifying this peptide has been described by Oyer et al (1971)

The assay is carried out by the double antibody method (Morgan and Lazarow 1963) and methods for measuring C-peptide in unextracted serum have been developed. Details of the procedure are available in several publications (Horwitz, et al 1975 b Block, et al 1972 a) Because the entire C peptide structure is present within the proinsulin molecule it is not surprising that proinsulin will cross react in the C-peptide assay With our present antiserum proinsulin displaces the tracer about 1/10 as well on a weight basis as C-peptide In serum taken from fasting healthy subjects C peptide is present in approximtely ten times higher concentration than proinsulin (2 ng/ml vs 0.2 ng/ml) Thus, in normal serum less than 1 % of measured C-peptide immunoreactivity is attributable to proinsulin For this reason the values have been referred to as C-peptide reactivity (CPR) rather than C-peptide In sera containing higher amounts of proinsulin the CPR level will not accurately reflect the C-peptide concentration In most sera, however the contribution of proinsulin to CPR is negligible compared to the variability of the method and may be disregarded.

2. Metabolism of C-peptide

Because the concentration of a hormone in peripheral blood reflects the net effect of its secretion and degradation interpretation of

serum C-peptide levels requires an understanding of both these parameters. Studies *in vivo* of bovine C-peptide in the rat have shown total metabolic clearance rate (MCR) of 4 6 ± 0.2 ml/min which is less than that of either bovine insulin (16.4 ± 0 4 ml/min) or proinsulin (6 7 ± 0.3 ml/min) (Katz and Rubenstein, 1973) The MCR appears to be independent of plasma concentrations over the range of 1 to 15 ng/ml. Renal removal of C peptide from the circulation accounted for 69 % of its MCR while the renal contribution to insulin metabolism was only 33 % (Fig. 23). This difference is attributed to the liver having a significant role in the catabolism of insulin but not C-peptide The kidney on the other hand, is the major organ involved in the metabolism of the C-peptide. The more rapid MCR of insulin compared to C-peptide is also apparent in man. Measurement of plasma disappearance half-times (T 1/2) in patients undergoing removal of insulin-secreting pancreatic tumors has indicated that the T 1/2 of endogenous C-peptide is 11 1 minutes, compared to value of 4.8 minutes for insulin (Horwitz, et al. 1973)

C-peptide together with insulin and proinsulin, is secreted by the pancreatic beta cells into the portal circulation and must pass through the liver before entering the peripheral blood In order to relate peripheral concentrations to beta cell secretion, simultaneously determined portal and peripheral blood levels were compared in subjects whose portal blood was obtained by umbilical vein catherterization prior to operation (Horwitz, et al. 1975 c). Following stimulation by intravenous glucose or arginine both insulin and C-peptide reached peak concentrations at 90 to 120 seconds after the onset of the stimulus, while the peak did not occur until 2 to 5 minutes in the peripheral circulation I portal blood, the relative increase over basal values for insulin was greater than that for C-peptide while in the peripheral circulation this difference was not as great. In general, both portal and peripheral serum concentrations of C-peptide were greater

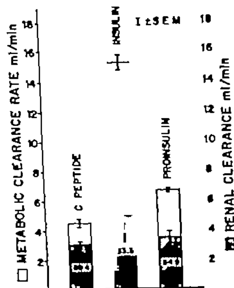


Fig 23
Renal clearance and metabolic clearance rate of C-peptide insulin and proinsulin Although the renal clearance of the three polypeptides was similar its contribution t the total M C.R (figures in cross-hatched areas) varied (Reproduced from Katz and Rubenstein 1974)

than those of insulin on a molar basis, but at times of peak secretion insulin and C-peptide were present in portal blood in nearly equimolar quantities.

Because insulin and C-peptide are present in the beta cell in equimolar concentrations and are secreted in this ratio it was somewhat unexpected to find peripheral molar concentrations of C-peptide substantially greater than those of insulin (approximately 0 7 pmole/ml C-peptide compared to 0.07 pmole/ml insulin). However the slower MCR of C-peptide together with the greater hepatic extraction of insulin, provides an adequate explanation for this discrepancy The finding of equimolar portal concentrations of insulin and C-peptide during times of peak beta cell secretion, when recirculation is assumed to

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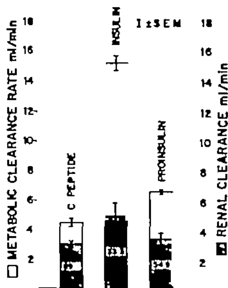


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contribute little to the measured concentrations of the peptide confirms that C peptide and insulin are indeed secreted in equimolar quantities *in vivo*

3 Clinical significance of C-peptide measurements

A C peptide as a measure of beta-cell function

Because the insulin immunoassay cannot distinguish endogenously secreted insulin from exogenously administered bovine or porcine insulin in insulin requiring diabetic patients (Yalow and Berson, 1961) measurement of CPR has proven to be extremely helpful in assessing their beta cell secretory function. The poor cross-reactivity between human bovine and porcine proinsulins and C-peptides (Fig. 24) (Rubenstein et al 1970 b) has indicated that the assay for human C-peptide will not be affected by small amounts of these species of proinsulin which may be present as impurities in commercial insulin preparations (Steiner et al, 1968)

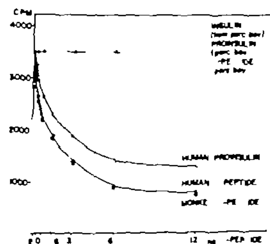


Fig 24
Reactivity of human monkey porcine and bovine insulin proinsulin and C peptides with a guinea pig antiserum raised to human C-peptide Only the human proinsulin and the monkey and human C-peptide competed with the ¹²⁵I tyrosylated human C-peptide tracer

A closely related situation occurs in patients who as a result of insulin injections, have developed circulating insulin antibodies. Although these antibodies may be of no clinical significance (except in patients with insulin allergy or insulin resistance) they interfere with the immunoassay of serum insulin. However because the antibodies do not affect the C peptide assay estimation of this peptide provides a measure of endogenous insulin secretion in these patients Nevertheless the results must be interpreted with caution because the circulating insulin antibodies may bind significant amounts of endogenously secreted proinsulin which cross-react in the C peptide assay (Fig. 25) (Block, et al. 1972 a & b) Exogenously administered porcine or bovine proinsulin occurring as an impurity in commercial insulin will not affect the result Measurement of CPR in these patients does serve as a qualitative indicator of beta cell function, but quantitative interpretation of the results is difficult because the catabolism of antibody-bound human proinsulin is markedly slower than that of C-peptide

B Sequential studies in newly diagnosed diabetic patients

Our understanding of the pathogenesis of diabetes mellitus has been enhanced by sequential studies of insulin levels in non-insulin requiring diabetic patients These studies have led to a greater appreciation of the natural history of the underlying beta cell dysfunction which characterizes this syndrome The C peptide immunoassay has permitted similar studies in insulin-dependent patients. Studies in adult-onset diabetics have shown that insulin and C-peptide levels are very low or unmeasureable during episodes of ketoacidosis Following recovery from ketoacidosis partial recovery of beta cell function occurs as assessed by increases in CPR (Block et al 1973 b) These studies indicate that diabetic ketoacidosis does not necessarily imply that irreversible beta cell damage has occurred, and suggest that functional impairment as well as cellular destruc

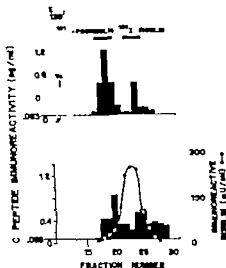


Fig. 25
Gel filtration of acid-ethanol extract of serum samples from a adult-onset insulin-requiring diabetic patient taken in the fasti g tat and two hours after glucose. The black bars indicate the results with the C-peptide assay (C-peptid tandard) and the II (o-o) shows the results with the insulin assay. Th column was calibrated with ¹³¹I-proinsulin and ¹³ I-insulin (top of figure). (Reproduced from Block et al 1972)

tion of beta cells may underlie this syndrome. This concept provides an optimistic basis on which to base future research initiatives.

Similar studies have been undertaken in patients with juvenile-onset diabetes mellitus (Block et al, 1973 b). The results have confirmed the absence of beta-cell secretion during periods of severe hyperglycemia and ketoacidosis. During the phase of clinical remission, the so-called "honeymoon period" or "Brush effect" C-peptide secretion may resume. Eventual clinical relapse in these patients is again associated with decline in serum CPR levels.

C-peptide measurements have also been useful in studying patients who have recovered from insulin-dependent diabetes. A particularly interesting example of such a patient with well documented mumps virus infection and diabetic ketoacidosis was described by Block, et al. (1973 a). Following clinical recovery from mumps her need for insulin gradually diminished with eventual return of normal carbohydrate tolerance. Her serum insulin could not be measured because of the development of circulating insulin antibodies but glucose and CPR responses were normal in both standard and cortisone-primed glucose tolerance tests (Fig. 26).

C Studies in established diabetes

The ability to measure serum C-peptide has for the first time permitted the assessment of beta cell secretory capacity in insulin-requiring patients who have developed circulating insulin antibodies. Furthermore because C-peptide reflects only endogenou insulin secretion, withdrawal of exogenous insulin is not necessary before evaluation of beta cell function is undertaken. Beta cell function can be measured in the insulin-requiring patient without interfering with the daily insulin regimen. Moreover the effect of insulin administration on beta cell secretory patterns can be determined as well as the role of endogenous secretion in modifying the requirement for exogenou insulin.

The presence of residual beta-cell secretory activity has been demonstrated in some adult-onset (Block, et al. 1972 a) and juvenile-onset (Block, et al 1974 Kuzuya, et al. 1974) insulin-requiring diabetics. The CPR in these patients consists of both C-peptide and proinsulin. The proinsulin is largely bound to circulating insulin antibodies, accounting for the high proportion of proinsulin in the basal state. Following glucose however both C-peptide and proinsulin may rise indicating an appropriate beta cell response to the glycemic stimulus. Thus Block, et al. (197? a) showed that, following 100 gm oral glucose CPR in normal subjects increases from 1.3 ± 0.3 ng/ml to 4.4 ± 0.8 ng/ml at 60 mi-

contribute little to the measured concentrations of the peptide confirms that C-peptide and insulin are indeed secreted in equimolar quantities *in vivo*

3 Clinical significance of C-peptide measurements

A C peptide as a measure of beta-cell function

Because the insulin immunoassay cannot distinguish endogenously secreted insulin from exogenously administered bovine or porcine insulin in insulin requiring diabetic patients (Yalow and Berson 1961) measurement of CPR has proven to be extremely helpful in assessing their beta cell secretory function. The poor cross-reactivity between human bovine and porcine proinsulins and C-peptides (Fig 24) (Rubenstein et al 1970 b) has indicated that the assay for human C-peptide will not be affected by small amounts of these species of proinsulin which may be present as impurities in commercial insulin preparations (Steiner et al 1968)

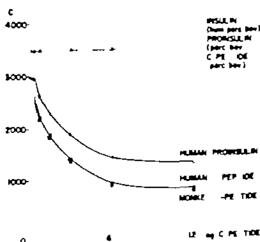


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disorders such as alcohol hypoglycemia. The C-peptide assay offers great potential in this regard.

Another difficult diagnostic problem in both diabetic and non-diabetic patients is distinguishing hyperinsulinism due to endogenous secretion from that due to the surreptitious injection of exogenous insulin. This distinction may be made by measurement of both IRI and CPR in serum taken when the patient is hypoglycemic. Because endogenously secreted insulin is accompanied by the liberation of C-peptide while exogenous insulin will suppress endogenous secretion and hence lower the serum C-peptide concentration (Horwitz, et al. 1975 a) serum IRI and CPR will show similar elevations in pancreatic hyperinsulinism while CPR will be low in relationship to the IRI following injection of exogenous insulin. This observation has been of great help in diagnosing several cases where the patients were surreptitiously administering insulin to themselves (Service et al 1975 Couropmitree et al 1975)

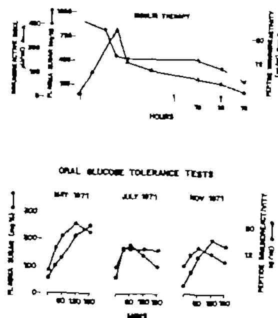


Fig 26
(Top) Sequential changes in levels of plasma sugar serum immunoreactive insulin and C peptide immunoreactivity before and during therapy for ketoacidosis Intravenous and subcutaneous porcine insulin administration resulted in raised serum insulin levels that were associated with a gradual decline in plasma sugar C-peptide immunoreactivity reflecting endogenous beta cell secretion remained undetectable throughout the episode (Bottom) Serum C peptide immunoreactivity and plasma sugar concentrations during renal oral glucose tolerance tests after the initial episode of ketoacidosis Although a mildly abnormal plasma sugar response was noted 8 weeks after the acute episode (May 1971) the oral glucose tolerance test was normal two months later (July 1971) and remained so during a cortisone glucose tolerance test 4 months later (November 1971) The use of C peptide immunoreactivity reflects the endogenous beta cell secretory response (Reproduced from Block et al 1973)

nutes while in adult onset diabetics CPR rose from a basal level of 2 7 ± 0 7 ng/ml to peak of 5 0 ± 1 0 ng/ml at 2 hours

In contrast, five ketosis-prone juvenile diabetics who had been treated with insulin for longer than five years had no measurable CPR in either the basal or post-stimulatory state Subsequent studies (Block, et al. 1974) however showed that CPR was present in a number of juvenile-onset patients during the first years of their disease This finding suggests that the loss of beta cell secretory capacity in *juvenile-onset diabetes is not abrupt*, but continues for several years after their diabetes becomes clinically manifest.

Comparative studies of stable and unstable diabetics have been facilitated by C peptide estimations. Adult insulin requiring patients were classified as stable or unstable on the basis of diurnal plasma and urine glucose variability The stable group had greater CPR levels in both the basal state and following stimulation with either oral glucose or arginine (Horwitz et al 1974) In the unstable group basal CPR levels were at the lower limit of sensitivity of the assay and no response to either glucose or arginine could be detected In addition these unstable diabetics also showed a severe alpha-cell defect as measured by minimal glucagon responses to hypoglycemia (Reynolds et al 1974) This suggests that diabetes mellitus may indeed be a bihormonal disease.

D Use of C-peptide in the diagnosis of hypoglycemic disorders

The diagnosis of hypoglycemic disorders has been greatly facilitated by measurements of serum insulin However difficulties arise in the evaluation of hypoglycemia in diabetic patients who have been treated with insulin injections for circulating insulin-antibodies in these patients interfere with the insulin assay In such patients *estimation of C-peptide* may be used instead of insulin to document endogenous hyperinsulinism (Sandler et al 1975) Therefore although insulinomas and nesidioblastosis are rare in diabetics they must be distinguished from other causes of spontaneous, fasting hypoglycemia such as mesenchymal tumors or hepatic or renal *failure as well as from non-insulin mediated*

disorders such as alcohol hypoglycemia. The C-peptide assay offers great potential in this regard.

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A glance to the future

1 The etiology of diabetes

Despite the very substantial progress that has been made in recent years in our knowledge of the insulin biosynthetic and secretory mechanisms of the beta cell we are still far from a complete understanding of the underlying pathological processes that lead to the development of diabetes in man With the development of the immunoassay for insulin it has become increasingly accepted that diabetes usually results from some degree of secretory failure of the pancreatic beta cells (see Chapter by Yalow this volume) In juvenile diabetics the extent of this failure is severe and is reflected in gross destruction of islet tissue In adult diabetics secretory failure is less pronounced but when patients are carefully classified so that variables such as obesity are controlled some degree of impairment of insulin secretion is almost always observed In the glucose tolerance test there is both a quantitative decrease in total insulin secretion as well as a sluggish early response with a tendency for the peak level to occur later than normal

The time course of the insulin response has been considered to provide significant clues to the nature of this defect (Cerasi and Luft this volume) Thus the delay in first phase insulin secretion (Curry et al 1968) generally correlates well with the tendency in these tests for the blood sugar level to rise to higher levels and to peak at later times It has been suggested on the basis of these results that the beta cells of diabetics may have an inherent or acquired alteration in their sensitivity to glucose as a stimulus This concept is supported by the observations that other stimuli to insulin secretion such as tolbutamide and glucagon elicit normal secretory responses in mild diabetics at a time when the response to glucose is already impaired (Simpson et al 1968)

Studies of the pathologic changes in the pancreatic islets in adult onset diabetes support the concept of a primary failure of beta cell responsiveness Gepts (197?) has pointed out that there is almost invariably a reduction in total islet tissue mass in the diabetic pancreas amounting to approximately 50 per cent in many cases Moreover there is a reduction of insulin stores in the surviving pancreatic beta cells of these individuals as evidenced by partial degranulation of the islet tissue and by a decrease in the total insulin that can be extracted from the pancreas. These pathological data suggest that the defect may involve the production of insulin and the regeneration of beta cells as well as the secretion of the hormone It is interesting to hypothesize that these defects may have a common and interdependent origin in the context of an altered glucose receptor mechanism in the diabetics beta cells. Such an alteration could possibly contribute to the impaired renewal of islet tissue through failure to adequately stimulate cell division for there is evidence that hyperglycemia may play a role in stimulating mitotic activity in the islets (Andersson 1975) In addition, this failure to respond normally to glucose could lead to a decrease in insulin biosynthesis and storage inasmuch as glucose concentration is a potent stimulus to these processes Finally the failure of an adequate mechanism to monitor extracellular glucose concentration would of course result in impairment of insulin secretion

Although it is possible that the beta cell defect(s) in diabetes may involve only the efferent component of the insulin release mechanism i e Ca dependent granule extrusion processes one would anticipate that the cellular stores of insulin would not only be preserved but might be even greater than normal under these conditions One might also anticipate that islet cell regenerative activity

would lead to beta cell proliferation and islet hyperplasia. This situation has indeed been found to occur in the diabetic spiny mouse (Stauffacher et al 1970), where there is a great increase in total islet tissue mass and the beta cells contain numerous secretion granules and large quantities of insulin. Recent evidence has indicated that these animals have an intrinsic defect in the insulin secretory mechanism, probably involving the microtubular-microfilamentous system. The contrast between this animal model and the known beta cell pathology of human diabetes described above strongly favors the hypothesis that faulty metabolic or receptor recognition of glucose may underlie the human disorder in many cases.

But while the concept of a genetically determined intrinsic defect in the beta cells of most diabetics is an attractive working hypothesis other factors of largely environmental origin, merit further consideration. Recent studies of the inheritance pattern of diabetics and its incidence particularly in identical twins (Tattersall and Pyke 1972) strongly suggest that other causes for diabetes may exist and account for significant fraction of the total number of patients. Studies of pancreatic pathology particularly in juvenile diabetics, indicate the occurrence of a complex destructive lesion in the islets of Langerhans that may be due to extrinsic causes acting in genetically favorable situation. Among such cases are two of particular interest and concern, autoimmunity and viral infection. These subjects have been dealt with in detail elsewhere (Bastenie et al 1974 Craighead 1972).

Although there is little evidence at present to support the idea of defect of the receptor for insulin in diabetes, recent studies with lymphocytes have indicated that abnormalities in membrane-binding of insulin may occur under certain conditions (Olefsky and Reaven, 1974). Elucidation of the important structural features of insulin necessary for its biological action as well as the associated molecular events are both of obvious importance to the complete understanding and successful treatment of diabetes.

2. Fundamental research in diabetes

Although, as discussed above, there is considerable evidence that abnormalities in beta cell structure or function may be etiologically involved in the expression of diabetes there is not, as yet absolute certainty on this point. Consequently our experimental attack on the problem of diabetes must be carried out on a very broad front. The development of more basic knowledge concerning the regulatory mechanisms of the beta cells their growth replication, and biosynthetic processes, as well as their secretory mechanisms is of utmost importance. Moreover it is impossible to predict how new information derived from other areas of basic biological investigation may change our concepts regarding the causes of diabetes. It is quite probable that no one single defect underlies all cases of diabetes. Many diverse abnormalities in beta cell integrity may result in a clinical syndrome characterized by carbohydrate intolerance. Moreover both genetic and environmental factors may interact to alter beta cell function. Until all these ramifications are dissected out, it is essential to pursue both basic as well as clinical research in many areas.

Following are several areas of intensive fundamental investigation in which important breakthroughs may be anticipated in the years ahead.

(a) The natural history of the beta cells. We know relatively little about the normal life span and mitotic potential of beta cells and the factors which may influence or initiate their replication. Moreover we lack methods to study this important parameter in man. The ability to maintain islets and beta cells in organ and monolayer culture has begun to develop in recent years (Andersson and Hellerstrom 1971 Murrell et al. 1966 Sando et al 1972 b Macchi and Blumstein 1969

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3 New insulins for diabetes therapy

Considerable progress has been made in recent years in the quality of commercially available insulin preparations as reviewed in the chapter of Deckert and Poulsen in this volume. These advances have come largely as a consequence of the development of new concepts regarding the biosynthesis of insulin, i.e. the identification of proinsulin and related forms as well as through the application of newer techniques for the purification of proteins to the large scale production of insulin. These new insulin preparations are sufficiently pure and stable for example that they could be used in implantable metering devices the so-called "artificial pancreas" currently under development in several laboratories. These devices would presumably contain a sufficient supply of insulin to last for many weeks or months without requiring replacement. Since such devices avoid the use of long-acting insulin preparations which are generally more antigenic than soluble preparations it is likely that the problem of immunogenicity would be greatly ameliorated by a combination of the improved insulins with such metering devices

However problems of supply and demand overshadow the continued use of insulin as therapy for diabetes The widespread distribution and high level of incidence of diabetes poses the threat of an insulin shortage as the world population increases Thus at some future time the world's supplies of insulin may no longer be sufficient to meet the increased demand and some form of synthetic hormone will be necessary

Recent experiments of Brandenburg and Wollmer (Brandenburg and Wollmer 1973 Wollmer and Brandenburg, 1974) have led to the development of a promising new approach to the chemical synthesis of insulin which is based on the concept of a proinsulin-like molecule or "miniproinsulin" in which the A and B chains are efficiently combined by a small non-peptide connecting moiety which guides the correct folding and disulfide bond formation. These workers have found that the C-peptide can be replaced by a simple molecular cross-link between the epsilon amino group of the B-29 lysine and the alpha amino group of the A 1 glycine residue. Such cross-linked insulin molecules can be reduced under denaturing conditions and will subsequently regain native structure with correct disulfide bond formation just as in the case of proinsulin (Brandenburg and Wollmer 1973 Steiner and Clark, 1968). Busse, et al (1974) have devised a highly suitable cross-linking reagent for this purpose which can readily be removed by treatment with cyanogen bromide without affecting the insulin moiety (Fig. 27) These new synthetic methods could facilitate the chemical synthesis of insulin at lower cost by considerably enhancing the yields of active material obtained while still permitting the synthesis to be accomplished *via the separate synthesis of* the A and B chains. There are still however many problems in the synthesis of the A and B chains which must be solved before insulin can be synthesized commercially at a reasonable cost.

The successful use of the "miniproinsulin" for insulin synthesis suggests the possibility that other complex polypeptides might also be built up in the laboratory without the need for synthesizing entire peptide sequences Application of similar techniques to a variety of enzymes and other active proteins as for example growth hormone, might facilitate the synthesis of miniproteins which if properly designed, would retain many of the biological properties of the original protein molecules. If this can be accomplished, then the study of insulin will have pioneered yet another potentially fruitful research area in protein chemistry

In keeping with the above suggestions it is also possible to imagine that as progress of our knowledge of the binding interaction between insulin and its receptor protein(s) continues to develop in the next few years, it will become increasingly clear which regions in

Chick et al 1975) and promises to become one of the most important methods for pursuing these questions in the future With progress in maintaining and regulating beta cells in culture it may become possible to transplant islet tissues as a means of effective long term therapy The present status of pancreatic and islet transplantation research is hopeful but still presents many formidable problems (Goetz, 1974)

(b) The origin of the pancreatic islet cells Do beta cells arise from proliferating pancreatic ductules which are derived from the original embryonic duodenal diverticula (Wessels and Evans 1968) or are these derivatives of neural crest cells (Pearse et al 1973)? Do diabetic patients have a normal complement of beta cells before their disease becomes manifest? These questions will require additional quantitative morphological and functional studies on beta cells at various stages of development in both animals and humans

(c) Enzymic factors regulating insulin biosynthesis Isolation and characterization of the converting enzymes which transform proinsulin to insulin is necessary for a complete understanding of insulin formation. The existence of the precursor form of insulin has provided an enzyme mediated step in biosynthesis where inherited defects could occur (Steiner et al 1972) Although initial studies in diabetic patients have failed to support the hypothesis of a defect in conversion of proinsulin to insulin it is likely that abnormalities in this area will eventually come to light as more detailed studies are made

(d) The structures of proinsulin and insulin in diabetics Some genetic defects in insulin production may occur through point mutations in the structural gene for proinsulin or in related regulatory genes. Amino acid substitutions in the connecting peptide region of proinsulin could distort the folding of the peptide chain as to reduce the effectiveness of disulfide bond formation while alterations in the amino acid sequence in the regions of proinsulin cleavage could also lead to abnormal products Of course mutations within the insulin chains themselves could result in biologically ineffective insulin molecules. Some progress has been made in refining techniques for extracting insulin related proteins from single human pancreata, and for determining their amino acid sequences using very small quantities of material (Peterson et al 1972 1975) but much further work remains to be done in this area. Preliminary studies of proinsulin C-peptides from 5 diabetic pancreata obtained at necropsy indicating no gross changes in composition or electrophoretic behaviour (Peterson Nehrlich and Steiner unpublished data)

(e) The molecular biology of insulin production As discussed earlier (Sect 3 Part 3) development of techniques for the isolation of the messenger RNA for proinsulin (See Fig. 13) will provide the means to study the proinsulin mRNA per beta cell under various conditions There also are many ancillary questions of considerable theoretical significance relating to the structure of the proinsulin mRNA i e its relative size special nucleotide sequences involved in its translation and its content of reiterative sequences such as poly A (Lee et al 1971)

(f) The regulation of insulin biosynthesis and secretion It is of critical importance to resolve the issue of whether a structural glucoreceptor is present in beta cells and to determine its structure and properties if it can be found This problem will require extensive studies on the morphology and chemistry of the beta cell membrane and the correlation of these results with its biochemical properties. The development of suitable methods for isolating islet cell plasma membranes from rat islets is an important prerequisite for studies of this kind (Lernmark, et al 1975) Identification of beta cell plasma membrane components will also be of considerable importance from the standpoint of studies on histocompatability antigenicity and viral susceptibility

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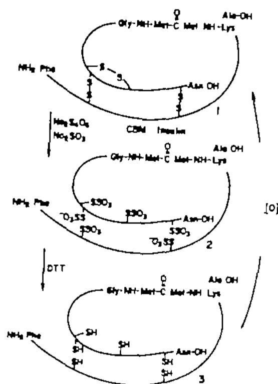


Fig 27
Diagram of the oxidative sulfitolysis of carbonylbis-(methionyl) insulin (CBM-insulin) 1 followed by the reduction of the S-sulfonate 2 with dithiothreitol (DTT) to yield the sulfhydryl form 3 which in turn is oxidized in air to yield the CBM-insulin (Reproduced with permission of authors from Busse et al 1974)

the three-dimensional insulin structure (Blundell et al 1972) are required for biological activity and in fact the precise chemical topography necessary for activity will be elucidated It may then become possible to create relatively small non-protein substitutes for insulin which have the appropriate molecular configuration to bind and modify the insulin receptor site Such analogs might be constructed from peptide fragments linked by non-peptide material or could consist entirely of non-peptide material Such substances would not only be more stable than insulin (some of them might even be too stable biologically because they might not be degraded readily in the organism) but they might also be capable of being efficiently absorbed after oral ingestion The attractive feature of these compounds as oral therapy for diabetes would derive from the fact that they would have a true insulin-like action and their uptake from the intestine would make it possible for them to act first on the liver as is also the case with endogenously secreted insulin Presumably these compound like many drugs presently used in medical therapy would not be antigenic for most individuals, or their antigenicity could be modified by careful chemical manipulation Thus advances in our understanding of the chemistry and biosynthesis of insulin resulting from the efforts of many scientists throughout the world may ultimately provide highly ingenious solutions for the important problem of provision of adequate supplies of insulin for therapy of diabetes. Hopefully however advances keeping pace in other areas of basic research on the islets of Langerhans will eventually obviate the need for insulin altogether

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Insulin secretion and the development of diabetes mellitus in the adult

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Contents

Introduction

Most authors presently agree that manifest diabetes mellitus both of the juvenile and mature-onset types, is accompanied by a deficiency in insulin secretion. Diverging views prevail however regarding pancreatic function in the initial stages of the disease. Thus some authors deny the existence of β-cell deficiency early in diabetes and assign a major pathogenetic role to the existence of peripheral resistance to the action of insulin. Others have proposed that the secretory capacity of the islets may decrease with time until exhaustion of the β-cells and appearance of diabetes.

Our work during the last decade has focused attention to the possibility that a functional modification of the β-cell may be present from early lif on and constitute one of the basic requirement for the development of diabetes of the maturity-onset type. A to juvenile diabetes increasing evidence suggests that viruses or autoimmunity may play a dominating pathogenetic role (Gamble et al 1973 Nerup et al. 1974 b Bottazzo et al 1974)

The present review which has the aim of presenting our personal opinion about the pathogenesis of maturity-onset diabetes, will deal mainly with the hypothesis of a primary β-cell deficiency Most of the discussion will be centered on the mechanism of insulin secretion partly because we consider the dysfunction of the β-cell to play key role in the development of the disease and partly because the technical advancement of recent years hav permitted detailed studies of islet physiology

Insulin secretion

Insulin is certainly one of the most important hormones controlling the glucose homeostasis. Among the numerous hormonal feedback loops of the organism the glucose-insulin system presents one with very tight coupling between stimulus and effector Thus, small changes in the blood glucose concentration are rapidly recognized by the pancreas which then immediately releases adequate amounts of insulin into the circulation. The physiologic importance of this tight coupling is illustrated by the fact that, a will be discussed later even minor delays and losses of sensitivity in the loop result n deterioration of the glucose tolerance of the organism.

1 Characteristics of glucose-induced insulin secretion

One main feature of the response of the pancreatic islet to hyperglycemia is its rapidity In the most sensitive assay systems such as in glucose infusions performed in vivo with frequent sampling from the portal vein (Blackard and Nelson, 1970) or in vitro using the perfused isolated pancreas preparation (Grodsky et al. 1968), it can be shown that a near-maximal rate of insulin secretion may be attained within seconds to a few minutes after raising the glucose concentration in the extracellular space (Fig. 1) The magnitude of this initial response is proportional to the concentration of the stimulus over a wide range of glucose levels, and demonstrates the usual sigmoid type of dose-response curve (Fig. 2).

A second typical aspect of insulin release concerns the marked changes that occur with time in secretion rate if the stimulation is maintained. Indeed the abrupt initial response is of short duration (5-7 min), the rate of

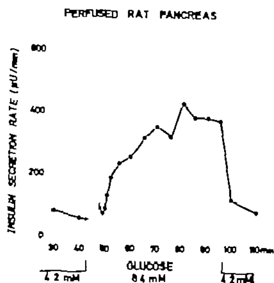
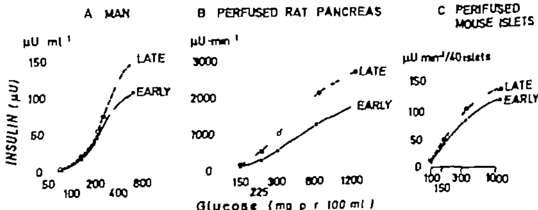


Fig 1
The pattern of insulin release from the perfused isolated rat pancreas The pancreas of a non-fasted rat was equilibrated during 42 min with a basal concentration of glucose (4.2 mM) in the perfusate Between 42 and 97 min the glucose concentration was doubled abruptly The initial peak value was obtained 2 min after raising the glucose level to 8.4 mM

secretion falling rapidly to a nadir which may be near the basal values This initial spike is later followed by a gradual increase in the release of insulin thus giving its typical biphasic appearance (Fig. 1) The same feature may also be observed in man in peripheral venous blood but with a different time scale due to distribution of insulin in its various body spaces (Cerasi and Luft 1963) A biphasic response is not an artefactual phenomenon due to rapid and unphysiologic elevation of the glucose level but represents an inherent characteristic of the β-cell since under appropriate conditions it may be observed even if the blood glucose is elevated gra

Fig 2
Similarity of the affinities for glucose of the early and late insulin responses
A Peripheral plasma insulin response to glucose infusion (priming and constant infusion) in healthy subjects Early response corresponds to the 10 min values the late one to the 60 min levels
B Insulin secretion rates from pancreas isolated from fasted rats stimulated by a square-wave pulse of glucose Early response is at 2 min the late one at 40 min after having increased the glucose concentration of the perfusate from 80 mg/100 ml to the respective values (data from Efendić et al to be published)
C Insulin secretion rates of perifused isolated mouse islets Legend as for B except that early response is at 5 min and the late one at 30 min Basal glucose concentration in the perfusate was 50 mg/100 ml (data from Rabinovitch et al to be published)



dually and to a modest extent (Cerasi et al. 1973 b).

Two types of explanations have been proposed for the biphasic pattern of insulin release. According to the more established view insulin is segregated into two compartments within the β-cell (Cerasi 1967 Grodsky et al 1970 Grodsky 1972) These compartments may be visualized either as two distinct functional units, or as corresponding to an anatomical reality (e.g. insulin granules located near the cell membrane, and those present in the more central parts of the cell) The first compartment, which is presumed to be of limited size reacts rapidly on stimulation, and discharges its content to the extracellular space within a duration corresponding to the initial peak depicted in Fig. 1 The second compartment has slower kinetics but a larger capacity and constitutes the later insulin response.

Attractive as it may seem the compartmental model of insulin secretion is insufficient to account for several aspects of insulin secretion. Most important as apparent from Fig. 2, the dose kinetics of the initial response is not different from those of the late one saturation being approached at very high glucose concentrations only This indicates that the reason for the initial limited response cannot be the exhaustion of insulin in small pool or the extrusion of all granules present at the cell periphery If this was really the case, biphasic responses would be obtained only above a certain threshold of stimulation which surpasses the capacity of the small compartment and no dose-effect relationship would exist for the initial pike above that threshold. For these reasons, we have proposed another model which does not necessitate the postulation of anatomical insulin compartments, but which assumes that positive and negative feed-back loops control the effect of glucose on insulin release and that due to differences in the time kinetics of these loops, the final response receives it biphasic appearance (Cerasi et al. 1974).

2. Multiplicative model for glucose-induced insulin release

In this model it is assumed that when glucose reaches the β-cell, three events are generated almost simultaneously although their expressions (especially for maximal efficiency to be reached) necessitate different time lapses (Fig. 3) It is suggested that two distinct recognition sites for glucose give rise to the two positive inputs in the β-cell. *Initiation* of the signal for the firing-off of insulin which has a very rapid course and lasts as long as the glucose concentration in the extracellular space is elevated and *potentiation* which is a concentration and time-dependent state of enhancement that results in amplification of the former effect of the sugar The markedly different time-courses of these two events are schematically illustrated in Fig. 3 The third event which has an intermediary time course, corresponds to the negative *feed back regulation* of insulin release and, as will be discussed later may be generated either by the phenomenon of release as such or

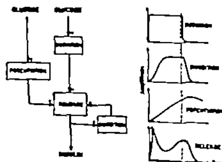


Fig 3
Effect of glucose on the activation of insulin release It is suggested that two positive inputs (initiation and potentiation) generated by glucose and one negative input (inhibition) generated by insulin itself or some process linked to it release modulat the final response of the pancreatic islet The time-courses of these events at the β-cell are thought to be strikingly different This is schematically illustrated on the right-hand panel of the figure (From Cerasi 1975 f)

induced by some effect on the β-cell of the insulin that has been secreted. It is postulated that the superposition of these three events with different kinetics results in the typical biphasic insulin release pattern (Fig. 3) Based on these ideas a mathematical model for the regulation of insulin release (and blood glucose homeostasis not shown here) was developed The insulin secretion rate can be described by the following set of equations

$$\frac{di}{dt} = k_i \; i_r \qquad (1)$$

where K_i is a parameter of individual sensitivity to glucose (in $\mu U ml^{-1} min^{-1}$) and the dimensionless variable i_r an abstraction representing the dynamics of insulin release

$$i_r = e^{p-b} f(g) \qquad (2)$$

The non-linear function f(g) describes the influence of varying glucose levels on initiating the insulin secretion and results in a sigmoid type dose-response curve for insulin release. The potentiation induced by glucose is described by e^p and the feed-back inhibition of insulin release by e^{-b}

$$\frac{dp}{dt} = (1/\tau p)\ (p-p_1) \qquad (3)$$

$$\frac{dp_1}{dt} = (1\ 1/\tau p\ )\ p_1 + k_p\ \ f(g) \qquad (4)$$

Thus the potentiator variable p is a function of f(g) and K_p (a parameter of individual sensitivity) and has a complex course over the intermediate variable p_1 The time constants $\tau p = \tau p_1$ were set at 30 min Variable p enhances insulin secretion in a multiplicative manner as the power of e

The negative feed back control of insulin release may be described in a similar manner

$$\frac{db}{dt} = (1/\tau b)\ (b-b_1) \qquad (5)$$

$$\frac{db_1}{dt} = (1/\tau b\ )\ b_1 + k_b\ i_r \qquad (6)$$

Thus the inhibitory variable b is generated over the intermediate variable b by the release process i_r, parameter K_b defining the sensitivity of the individual As for the potentiation the feed-back inhibition is visualized as a multiplicative e^{-b} function. The time constants used were $\tau_b = 5$ min, $\tau_{b1} = 20$ min.

The parameters K_i K_p and K_b (together with other parameters concerning the glucose part of the model not shown here) were individually adjusted during the computations Fig. 4 gives an example of the computer analysis of these different functions based on a glucose infusion experiment performed on a healthy subject.

In the following, the physiologic and biochemical basis for the above described phenomena of initiation, inhibition and potentiation will be discussed in some detail

3 Initiation of insulin release by glucose

The rapidity by which the pancreatic islet increases its rate of insulin secretion when stimulated suggests that the chain of events involved in the recognition by the β-cell of changes in the extracellular concentration of glucose in transmission of this message to intracellular sites the movement of insulin granules to and/or fusion of the granular membrane with the cell membrane and finally extrusion of the granules to the extracellular space by emiocytosis must proceed at a very high speed The cellular unit responsible for recognition of glucose as an initiator of these processes has been investigated actively during recent years Unfortunately the bulk of the existing data stems from experiments in which the kinetic peculiarities of insulin secretion were totally ignored Thus, the well established view that accumulation of a product of glycolysis in the β-cell may initiate release was based on correlations obtained between various parameters of glucose utilization on one hand and insulin secretion on the other in islet incubations of 60-120 min duration where the data were col-

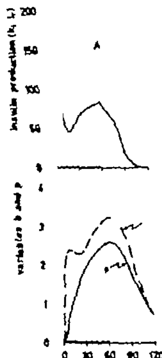


Fig. 4
Simulation of the effect of hyperglycemia on the generation of potentiation (p) and inhibition (b) the islets nd the secret on rate of insulin (k) Gl se was administered in the actual experim nt as priming injection followed by constant rate infusion during 60 minutes (horizontal bars on th figure) (From Cerasi 1975 f)

lected in a cumulative manner (Hellman, 1970 Ashcroft et al 1970 Lambert, 1970)

For this and other reasons it has been questioned whether insulin release may not be activated by some direct action of glucose prior to its metabolism and eventual accumulation of metabolites in the β-cell.

4 The β-cell glucoreceptor"

Cerasi and Luft (1970 b) and Matschinsky et al (1971) proposed that glucose as such might bind to cell membrane receptor which then transmits the information to the insulin release mechanisms If such a glucoreceptor operates in the islets, the initial insulin response to glucose might be dissociated from the early metabolic effects of the hexose in the β-cell. Experiments in which the intracellular accumulation of glucose metabolites were measured under dynamic conditions, failed to give a definite answer to this question (Matschinsky et al. 1972 Idahl 1972 Hellman et al 1974).

However recent findings with the use of the two anomeric forms of D-glucose have given support for the glucoreceptor hypothesis. Thus insulin secretion is stimulated preferentially by the α-anomer of glucose (Niki et al 1974 Grodsky et al. 1974) In constrast the islet like other tissues seems to metabolize preferentially the β-anomer which on the other hand, has a lower insulinogenic capacity (Idahl et al. 1975) Although this functional distinction between the α and β-anomers of glucose is not an absolute one it does suggest that the β-cell has developed the specialized property of recognizing the α-anomer as the trigger for the insulinogenic signal

It may be questioned whether the initial and late insulin responses to glucose have different mechanisms e g. the initial one being controlled by a glucoreceptor the late one more influenced by β-cell glucose utilization. If one considers the glucose dose-response characteristics of the initial and the late insulin responses however it may be seen that the k_m for these two functions are similar both in man and in a variety of experimental models (Fig. 2), while the capacity (V_{max}) of the late response is usually somewhat greater Thus, whatever cellular mechanisms are involved in these two phases of insulin release their affinity to glucose seems to be identical It seems therefore more logical to suggest, as in our mathematical model that the action of glucose on the initiation of insulin release is uniform with time, only one recognition unit being involved

induced by some effect on the β-cell of the insulin that has been secreted. It is postulated that the superposition of these three events with different kinetics results in the typical biphasic insulin release pattern (Fig. 3) Based on these ideas a mathematical model for the regulation of insulin release (and blood glucose homeostasis not shown here) was developed The insulin secretion rate can be described by the following set of equations

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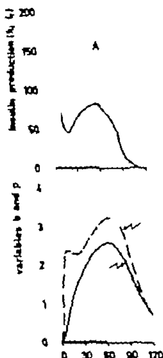


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specifically raises the intracellular cyclic AMP levels in the islets. Under some of our incubation conditions, this effect seems to precede the release of insulin (Grill and Cerasi 1974)

How does glucose influence the cyclic AMP levels in the islets. Present evidence does not allow the selection of one (or combination) of the following possible mechanisms, direct stimulation of the adenylate cyclase enlargement of a specific ATP pool serving as substrate for the cyclase inhibition of the phosphodiesterases. Although attempts have been made to measure some of these steps none can be excluded at present (Sams and Montague 1972 Howell and Montague 1973). It is likely that the postulated glucoreceptor is involved in the transmission of the glucose effect to cyclic AMP Indeed in analogy with the findings concerning insulin release, it was demonstrated that islet cyclic AMP is preferentially stimulated by the α-anomer of D-glucose (Grill and Cerasi 1975)

The question may be raised as to whethe the effects of glucose on cyclic AMP on the one hand, and on insulin release on the other are causally related. Fig 6 attempts to answer this question by showing the correlation that exists between the release of insulin and outflow of labelled cyclic AMP into the medium over 60-min incubation of rat islet in the presence of varying glucose levels (Grill and Cerasi 1974) Accumulation of cyclic AMP in the medium rather than in the tissue was selected since only the former is a cumulative parameter comparable t the accumulation of insulin The correlation between the two parameters is striking, the cyclic AMP and insulin responses to glucose sharing identical K_m values This finding suggests, but certainly does not prove that the recognition unit of glucose for insulin release and that for activation of cyclic AMP accumulation, may be identical I other words, cyclic AMP may indeed be mediating the glucose action to the site of insulin release

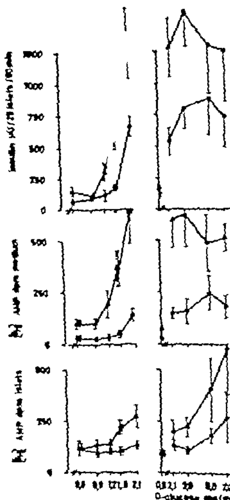


Fig 6
Effect of different concentrations of glucose on accumulation of cyclic AMP in rat islets efflux of the nucleotide into the medium, and ecretion of insulin over 60 min incubation. Triangles denote incubations in the presence and circles in the absence of 0 1 mM IBMX (From Grill & Cerasi 1974)

In conclusion, we feel that present experimental evidence strongly indicates that the effect of glucose on insulin release in the β-cell is mediated by changes in the cyclic

Once the increase in extracellular glucose concentration is recognized by the β-cell how is this information transmitted further to the insulin release organelles? As in many other cell systems cyclic AMP seems to play the role of the second messenger also in the pancreatic islets

b Cyclic AMP and insulin release

It has been known for some years that the agents which supposedly increase the β-cell cyclic AMP content also enhance the secretion of insulin (Malaisse et al 1967 Turtle and Kipnis 1967 Lambert et al 1971) Therefore it has been accepted that the adenylate cyclase-cyclic AMP system of the β cell may be involved in insulin release Based on a number of indirect evidences we proposed some years ago that the simplest way of interpreting the data was to assume that glucose acts by increasing the levels of cyclic AMP in the cell (Cerasi and Luft 1970 b) The verification of this hypothesis has encountered difficulties due to the technical problems involved in measuring the minute amounts of cyclic AMP present in the isolated islets of Langerhans However recently we as well as others have succeeded in demonstrating that glucose indeed augments the islet cyclic AMP (Grill and Cerasi 1973 1974 Charles et al 1973) Our results will be summarized in the following.

The ATP pool of isolated rat islets was labelled by preincubating the tissue with ^{3}H adenine and the rate of conversion of ATP to cyclic AMP was measured in a following incubation. This method gives a measure of the net accumulation of cyclic AMP (i e production rate minus rate of degradation) in the tissue or incubation medium It could be shown that seconds to a few minutes after raising the glucose concentration of the incubation medium an increase occurred in the islet content of cyclic AMP (Fig. 5) The action of high glucose on cyclic AMP accumulation was sustained throughout a 2 hr incubation there were no marked changes similar to those typical for the insulin secretion rate

Furthermore the glucose effect on cyclic AMP was rapidly reversible within 90 sec, either by lowering the glucose concentration or adding mannoheptulose even in the presence of a phosphodiesterase inhibitor Finally the L isomer of glucose was totally inactive and the accumulation of cyclic AMP in the rat adipocyte was not influenced by D-glucose Artefactual effects of glucose on the islet cyclic AMP measurements were excluded by the demonstration that also the total extractable cyclic AMP content of the tissue (as measured by the protein kinase binding assay) is augmented by glucose and that the hexose does not alter the specific radioactivity of the islet ATP pool Using the technique of perifusion of isolated islets placed in a small incubation chamber it could be shown that also the release of cyclic AMP from the tissue is rapidly stimulated by glucose (Rabinovitch et al 1974) These findings support our earlier hypothesis that glucose

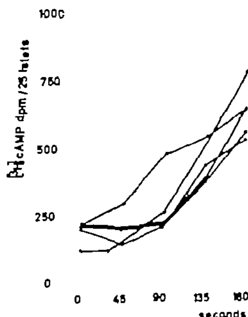


Fig 5
Time-course of the effect of glucose (5.0 mg/ml) on cyclic AMP accumulation in isolated rat islets (From Grill & Cerasi 1974)

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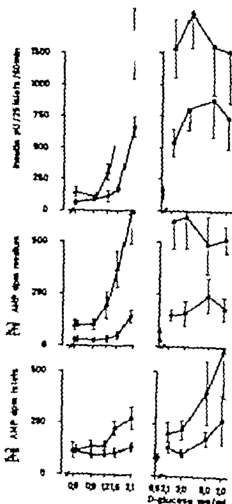


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b Cyclic AMP and insulin release

It has been known for some years that the agents which supposedly increase the β-cell cyclic AMP content also enhance the secretion of insulin (Malaisse et al 1967 Turtle and Kipnis 1967 Lambert et al 1971) Therefore it has been accepted that the adenylate cyclase-cyclic AMP system of the β cell may be involved in insulin release. Based on a number of indirect evidences we proposed some years ago that the simplest way of interpreting the data was to assume that glucose acts by increasing the levels of cyclic AMP in the cell (Cerasi and Luft 1970 b) The verification of this hypothesis has encountered difficulties due to the technical problems involved in measuring the minute amounts of cyclic AMP present in the isolated islets of Langerhans However recently we as well as others have succeeded in demonstrating that glucose indeed augments the islet cyclic AMP (Grill and Cerasi 1973 1974 Charles et al 1973) Our results will be summarized in the following.

The ATP pool of isolated rat islets was labelled by preincubating the tissue with ^{3}H-adenine and the rate of conversion of ATP to cyclic AMP was measured in a following incubation This method gives a measure of the net accumulation of cyclic AMP (i e production rate minus rate of degradation) in the tissue or incubation medium It could be shown that seconds to a few minutes after raising the glucose concentration of the incubation medium an increase occurred in the islet content of cyclic AMP (Fig. 5) The action of high glucose on cyclic AMP accumulation was sustained throughout a 2 hr incubation there were no marked changes similar to those typical for the insulin secretion rate Furthermore the glucose effect on cyclic AMP was rapidly reversible within 90 sec, either by lowering the glucose concentration or adding mannoheptulose even in the presence of a phosphodiesterase inhibitor Finally the L isomer of glucose was totally inactive and the accumulation of cyclic AMP in the rat adipocyte was not influenced by D-glucose Artefactual effects of glucose on the islet cyclic AMP measurements were excluded by the demonstration that also the total extractable cyclic AMP content of the tissue (as measured by the protein kinase binding assay) is augmented by glucose, and that the hexose does not alter the specific radioactivity of the islet ATP pool Using the technique of perifusion of isolated islets placed in a small incubation chamber it could be shown that also the release of cyclic AMP from the tissue is rapidly stimulated by glucose (Rabinovitch et al 1974) These findings support our earlier hypothesis that glucose

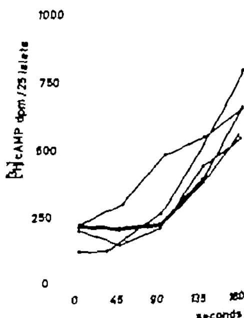


Fig 5
Time-course of the effect of glucose (5.0 mg/ml) on cyclic AMP accumulation in isolated rat islets (From Grill & Cerasi 1974)

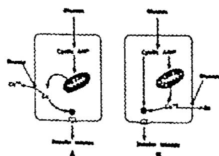


Fig 7
The interplay between cyclic AMP and calcium in the β-cell. Two possibilities are visualized. A – Sequential action. Glucose-induced cyclic AMP accumulation mobilizes calcium from intracellular stores (mitochondria etc.) which then activates insulin release. B – Parallel action. Cyclic AMP acts directly on insulin release, an action which is enhanced by the increase in cytosolic calcium level. Glucose also diminishes the efflux of calcium from the cell. (From Cerasi 1975 f).

tions must then be controlled at steps distal to cyclic AMP, i.e. by modulation of the effect of the signal of initiation on the mechanisms that effectuate the process of release. These modulatory events will be discussed below.

4. Feed-back inhibition of insulin release

As discussed above, we assume that during insulin secretion a phase of unresponsiveness or inhibition is rapidly created in the islet. What experimental evidence may support this assumption. Fig. 8 demonstrates that when two consecutive glucose challenges are given at short intervals in man, the plasma insulin response to the second stimulation is greatly diminished, while blood glucose usually shows higher values. Similar findings were also obtained in vitro with the perfused rat pancreas preparation (Grodsky et al., 1967). This phase of inhibition fades out relatively rapidly after cessation of the stimulation. The degree of inhibition of the second response in experiments like those of Fig. 8 was found to be significantly correlated to the magnitude of the first response (Cerasi 1975 a). This suggests that, as assumed in the mathematical model, inhibition is not generated by the hyperglycemia as such which was of similar magnitude in subjects with high or low insulin responses to the first challenge but by insulin release (I_s).

The mechanism of this feed-back inhibition is not known. It has been shown by some authors that exogenous insulin added to the perfusate of the pancreas, may diminish the secretion of the hormone (Iversen and Miles, 1971). Others disagree with these results (Grodsky et al. 1973). In man the parameter that showed the best correlation with the degree of inhibition was the insulinogenic index

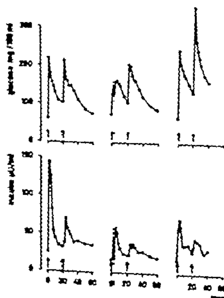


Fig 8
Feed-back inhibition of insulin release in man. Two consecutive glucose loads (15 g) were given intravenously at 20 min interval (arrows). In spite of higher blood glucose levels at the second challenge plasma insulin response was markedly diminished (results in three subjects). (From Cerasi et al. 1974)

AMP level of the cell The analogy between *the effect of* a number of hormones on their target cells and the action of glucose on the pancreas in this respect is striking, and assigns to circulating glucose apart from its obvious role of substrate that of a first messenger Clarification of how the β-cell has specialized to recognize glucose as a 'hormone' while still using it as a substrate must be a challenging task for comparative endocrinologists

c Calcium ions and insulin release

Another factor that plays a key role in secretory processes is calcium As with many other glands the release of insulin from pancreatic islets is abolished in the absence of a threshold concentration of calcium ions in the incubation medium. The presence of actin like material in the β-cell and possibly their discharge through the cell membrane by emiocytosis may be processes that are activated by calcium The evidence for the involvement of calcium in glucose-induced insulin secretion has recently been reviewed (Malaisse 1973) It has been shown that increasing the glucose concentration of the incubation medium reduces rapidly the efflux of radioactive calcium from islets and this has been accepted as evidence that glucose thus increases the cytosolic calcium ion level.

The question can be raised whether decreasing the outward transport through the cell membrane of calcium ions alone can augment their concentration in the cytosol Indeed it is known from both other tissues and the pancreatic islets that several subcellular organelles and especially the mitochondria, have a high affinity and capacity for calcium (Lehninger et al 1967 Hellman et al 1971). Thus release of calcium from intracellular stores probably has more profound effects on its cytosolic level and it has been suggested that cyclic AMP is one of the factors that liberate the ion from such stores (Rasmussen et al 1974) Evidence exists suggesting that cyclic AMP may have a similar effect on the pancreatic islet and that the amplifying action of e g theophylline on insulin release may be due to its mobilization of calcium from intracellular stores (Brisson et al 1971)

If one takes into account our demonstration of the effect of glucose on cyclic AMP levels in the islet the following hypothesis may be proposed for the glucose-induced insulin release (Fig. 7) Glucose augments rapidly the level of cyclic AMP which i.a releases calcium ions from sites where it is bound thus increasing its cytosolic levels. Another (independent?) effect of glucose limits the loss of cytosolic calcium through the cell membrane Insulin release is controlled either by calcium alone the sequence of events then being glucose → cyclic AMP → calcium or synergistically by cyclic AMP and calcium (permissive effects?) both agents acting in a parallel manner on secretion. Although recent findings demonstrating considerable insulin release by a calcium ionophore in the absence of glucose may support the first alternative (Zawalich et al 1974 Wollheim et al 1975) it has also been claimed that the ionophore increases the islet cyclic AMP content in glucose free medium (Zawalich et al 1975) Therefore much more experimental evidence is needed in order to clarify the relationship between cyclic AMP and calcium with regard to insulin secretion

From the above discussion it appears clearly that we visualize the signal of initiation of insulin release as a phenomenon involving the glucose recognition unit, transmission of its stimulation to the cyclic AMP system of the cell calcium ions and finally the effector organelles that discharge the granules of insulin The hypothesis presented in the mathematical model namely that the initiating action of glucose is uniform with time and lasts as long as the hexose concentration is raised is supported by the demonstration that cyclic AMP generation is continuously stimulated by glucose The changes seen in insulin secretion rate during such prolonged stimula

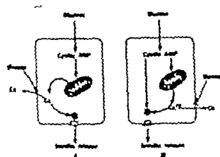


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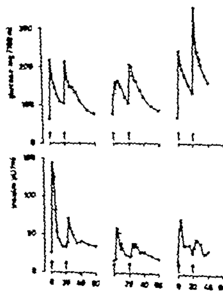


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of the first stimulus (i e the degre of stimulation induced by glucose) rather than the absolute levels of plasma insulin achieved during the challenge It is therefore conceivable that not the insulin concentration in the extracellular space but some function involved in the activation of insulin release by glucose generates the feed-back inhibition It must be emphasized however that the biochemical aspects of this feed back inhibition have not yet been studied

The teleological significance of this feed-back inhibition may reside in the fact that the action of the released insulin on blood glucose is relatively slow – in any case much slower than the action of glucose on insulin release By decreasing the secretion of insulin after the initial discharge during hyperglycemia time may be allowed for blood glucose to diminish thus preventing inappropriately high rates of secretion of the hormone which could lead to a later hypoglycemia.

5 Potentiation of insulin release by glucose

More important than the above short-lasting inhibition is the enhancement of insulin release that appears when the islets have been exposed to hyperglycemia for some time Fig. 9 shows a typical experiment in man where it is seen that both the initial and late phases of the insulin response to glucose are enhanced by previous administration of the hexose This synergistic effect of glucose on its own action was shown to be time dependent both in vitro (Grodsky et al 1968) and in man (Cerasi et al 1974) its time-lag in onset and its duration being considerably longer than those of the phase of inhibition

The exact time requirements for the building up of potentiation in the islets is not known but our studies indicate that a 20 min long hyperglycemic stimulus may be sufficient to enhance the next coming stimulations (Cerasi et al 1974) Under such conditions enhancement fades out when the time interval between two stimulations exceeds 60 min. The augmentation of the second insulin response reflects a true synergistic action between the glucose pretreatment and the acute stimulatory effect of glucose on insulin release (second stimulus) since the major part of the insulin response to the preinfusion of glucose had already disappeared at that time (Fig. 9). In experiments where the effect of a prior glucose load on the glucose-insulin dose-response relationship of the second challenge was evaluated it was shown that this synergism is of multiplicative type the threshold of

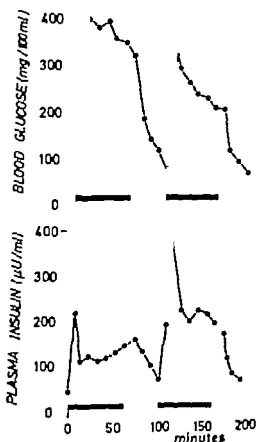


Fig 9
Enhancement of insulin response by pretreatment with glucose in man The horizontal bars on the abscissa symbolize two glucose infusions (500 mg/kg as priming and 20 mg/kg/min during 60 min) performed with a 40 min interval (From Cerasi 1975 f)

glucose for release of insulin is not modified but the response to higher doses is amplified, probably increasing the V_{max} of the response to glucose (Cerasi 1975 b) In this respect the enhancing effect of glucose on glucose induced insulin release is strongly reminiscent of the modulation induced by theophylline (Malaisse 1969) and arginine (Efendic et al 1974) but different from that due to tolbutamide which shifts the dose-response curve of glucose on insulin secretion to the left without modifying the maximum response to glucose (Loubatières et al 1970 Wahlström and Cerasi 1973 a). A major difference between the potentiation induced by glucose on the one hand and theophylline and arginine on the other is that while the latter agents act instantly as potentiators the effect of glucose is time-bound. Our studies suggest that maximum enhancement is induced by glucose after the end of a glucose infusion, the effect then disappearing with relatively long half-life (of the order of 40-50 min). The potentiation induced by pretreatment with glucose is not restricted to the insulin release induced by glucose but also to that in response to glucagon and tolbutamide administration (Cerasi 1975 c)

We have already suggested above that the potentiating action of glucose may be generated by other mechanisms than those generating the initiating signal of the sugar for insulin discharge Insulin ynthesis probably does not play major role in this context, since it has been demonstrated that newly ynthetized insulin (stimulated by glucose) is not released in significant amount before two to three hours of stimulation (Sando et al 1972, Sando and Grodsky 1973)

W postulated some years ago, and repeated this suggestion in our mathematical model, that glucose may play dual role in the pancreatic β-cell (Fig 10), and that the glucose utilization of the cell (e. glucose the substrate) may enhance the initiating action of the sugar (i.e glucose the signal) (Cerasi and Luft, 1970 b). Is there any evidence that initiation and potentiation of insulin release by glucose use different mechanisms in the islet We possess presently only indirect evidence suggesting that this may indeed be the case. Thus by using adrenaline to block insulin secretion we could demonstrate that normal release of insulin during administration of glucose is not a prerequisite for the generation of a state of enhancement in the islet (Cerasi 1975 c) Although insulin secretion during the glucose infusion was decreased markedly the responses obtained in the second stimulation were as high as when priming with glucose was allowed to occur with full insulin response. It is known from in vitro incubations of islets of Langerhans that the glucose utilization of the tissue is not influenced by the presence of concentrations of adrenaline much higher than those expected in our studies (Ashcroft et al. 1970). Therefore, it may be assumed that the catecholamine interferes with the insulinogenic signal of glucose rather than its metabolism. It is tempting to postulate that potentiation of insulin release by glucose which is not influenced by adrenaline, is a function linked to the metabolism of the hexose in the β-cell.

Another finding that strengthens the above suggestion is the marked difference that exists in the sensitivity of the islets to glucose regarding the acute insulin response on the one hand, and the generation of a state of potentiation on the other It could be shown that while release of insulin starts at a blood glucose level around 100-110 mg/100 ml (Cerasi et al 1972), potentiation of the next coming stimulation was obtained only if hyperglycemia around 250-300 mg/100 ml was achieved (Cerasi 1975 b) Thus, the dose-response curve describing the potentiating effect of glucose is shifted to the right of that of insulin release (see also Figs. 14 and 16). The teleological significance of this difference seems clear while the moment to moment regulation of the blood glucose level requires a system that is sensitive enough to register even small increases in glucose and responds immediately with an adequate release of insu-

of the first stimulus (i e the degree of stimulation induced by glucose) rather than the absolute levels of plasma insulin achieved during the challenge It is therefore conceivable that not the insulin concentration in the extracellular space but some function involved in the activation of insulin release by glucose generates the feed-back inhibition It must be emphasized however that the biochemical aspects of this feed-back inhibition have not yet been studied.

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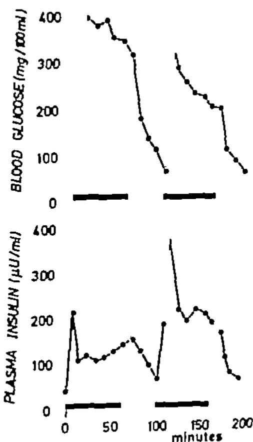


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completely obliterated in severe juvenile-onset diabetes, varying degrees of hypofunction are found in the milder maturity-onset type of the disease In patients with decreased glucose tolerance only as well as in those with overt diabetes mellitus the insulin response to glucose infusion shows a reduced or missing initial peak (Fig. 11) The later phase of the plasma insulin curve is either reduced and delayed or almost lacking. The completely flat insulin curves are predominant in juvenile diabetics but occasionally occur also in subjects who have decreased glucose tolerance only (Cerasi and Luft, 1967 a).

This depression in the secretory function of the β-cells in diabetics might indicate a progressive impairment in insulin release but may also be the expression of a genetic defect The former alternative has been proposed by Seltzer et al (1967). The latter implies that subjects liable to develop the disease, i e prediabetics would probably also have impaired insulin release In our studies, glucose infusions were performed on the only unequivocal group of prediabetic subjects the healthy members of monozygotic twin pairs in which the other member had diabetes (Fig 12) (Cerasi and Luft 1967 b). The insulin response was sluggish delayed and smaller than normal in this group thus of the type

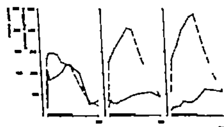


Fig 11
Glucose inf sion test in a high-insulin responder (A), low-insulin responder (B) nd diabetic (C) Solid curves denote insulin and broken curves blood glucos levels Glucose was infused betwee 0-60 min.

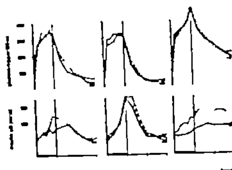


Fig 12
Glucose infusion test in three monozygotic twin pairs B oken line denotes diabetic sibling solid lin the healthy one Glucose infusion between 0-1 hour (From Cerasi & Luft 1967 b).

seen in subjects with latent or overt diabetes. In those instances where the glucose infusion test could be performed on both siblings of the monozygotic twin pairs the insulin responses were almost identical, whether both sibling were diabetics, one had overt diabetes and the other decreased glucose tolerance only or neither was diabetic. Hence it could be assumed that genetic factors determine the type of insulin response to glucose stimulation, and that the impairment of insulin release may be an inherited factor in diabetes mellitus

Recent findings support the above assumption (Lindsten et al 1976) The plasma insulin response to glucose infusion was measured in 52 non-diabetic family units consisting of the proband, his parents children and sibs. Furthermore, 24 monozygous and 29 dizygous like-sexed twin pairs were studied. The blood glucose and plasma insulin curves of each individual were used for analysis of the principal eigen-values, which were then used for the selection of compound variables characterizing the relationship between the glucose and insulin curves After correction for changes with sex, age and body weight and standardization with regard to mean and va-

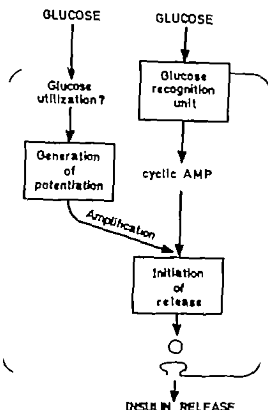


Fig 10
Hypothetical model for the β-cell recognition of glucose as an initiator and as a potentiator of insulin release See also text (From Cerasi 1975 f)

lin into the circulation amplification in the system will become necessary only if hyper glycemia of some importance persists for some time in spite of the acute insulin response As suggested by the studies of Porte and Pupo (1969) if its duration is augmented to several hours even a moderate to minor hyperglycemia may induce potentiation.

The above discussion suggests that both cellular units responsible for the recognition of hyperglycemia as a stimulus and the mechanisms that transmit further their actions in the β-cell are different for the initiating and potentiating activities of glucose The question that arises is the level at which enhancement of the insulin response occurs in the β-cell. Our demonstration that also the effects of glucagon and tolbutamide on insulin release are enhanced by glucose pretreatment indicates that the potentiation is not specific for the recognition of glucose (e g. alteration of the glucose recognition unit) (Fig. 10)

Is there any common mechanism in the stimulation induced respectively by glucose glucagon and tolbutamide which may hint at the (common) site at which the potentiating action of glucose may exert itself? It is accepted that glucagon releases insulin by acting on the adenylate cyclase of the β-cell (Turtle and Kipnis 1967) Both direct and indirect evidence suggests that tolbutamide may have an inhibitory effect on the phosphodiesterases of the islet cells (Goldfine et al 1971 Widström and Cerasi 1973 b) Thus in view of the demonstrated effect of glucose on cyclic AMP accumulation in the islet the action of all three agents may result in more cyclic AMP being generated in the β-cell It is therefore tempting to suggest that the potentiating action of glucose exerts its effect by modulating the influence of cyclic AMP on the insulin release mechanisms Evidently much experimental work is needed to assess the validity of this hypothesis

6 Insulin secretion in the diabetic syndrome

Diabetes mellitus is a disorder characterized by a decrease of the efficiency by which blood glucose homeostasis is controlled in man. The remaining degree of homeostasis defines the severity of the disease Thus a wide spectrum of diabetes-like states are encountered in clinical medicine from patients with severe hyperglycemia and ketosis to those who present normoglycemia at fasting but deterioration of the glucose tolerance i e relative hyperglycemia after meals or glucose challenges All the different stages of diabetes are accompanied by a reduction in the responsiveness of insulin release from the islets on stimulation by glucose (Yalow and Berson 1960 Seltzer et al 1967 Cerasi and Luft 1967 a) While insulin release may be

uniformity with time of the initiating action of glucose on insulin release we suggest that the recognition of glucose at any time during the stimulation is impaired in the diabetic.

The question may be raised whether this impairment is due to anatomical modifications in the β-cell (e.g. inability to extrude insulin granules diminishment in the amount of insulin that may be released etc.) or to changes in the transmission of the initiating signal of glucose. For this reason the second messenger of the signal i.e. cyclic AMP was measured in the islets of Acomys showing deficient release of insulin (Rabinovitch et al 1974). Similar techniques as those described above were used (p 116), and the efflux of insulin and cyclic AMP from perifused islets measured. Fig. 13 compares the result obtained in islets from rats and from Acomys. Basal release rates of insulin and cyclic AMP were not significantly different in the two species. When the concentration of glucose was raised from 2.8 to 16.8 mM there was an increased release of insulin and cyclic AMP from rat islets, but initially no significant effects on Acomys islets. Increased release of insulin and cyclic AMP from Acomys islets was delayed until the subsequent collection periods. After 15 min insulin release from Acomys islets attained levels about 75 % of those in the rat whereas cyclic AMP efflux from Acomys islet w only about 25 % of that in the rat. The decreased efflux of cyclic AMP from Acomys as compared to rat islets represents lesser formation of cyclic AMP in the former as evidenced by experiments in which the intracellular level of the nucleotide was also shown to be lower than in rat islets. These results suggest that, in the Acomys the defect in the transmis ion of the hyperglycemic stimulus is prior to the formation of cyclic AMP either at the recognition site of glucose or at the link( ) between this site and the adenylate cyclase system

Whether this finding applies also to the islet of the human diabetic is obviously unknown, although earlier findings with the use of theophylline may suggest the existence of an analogous situation in man. Indeed the low insulin response to glucose of some prediabetic individuals could be corrected by the administration of theophylline which, presumably amplifies the effect of glucose on cyclic AMP accumulation by blocking the breakdown of the nucleotide (Cerasi and Luft, 1969 a).

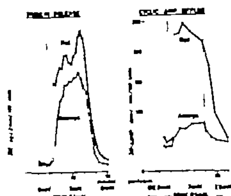


Fig 13
Insulin release and efflux of cyclic AMP from perifused islets of rat and Acomys The islets were preincubated for 60 min with H-adenine and then placed in perifusion chambers Two min pooled aliquots of the perifusate were purified as in Grill and Cerasi (1974) and the radioactivity of cyclic AMP measured (data from Rabinovitch et al 1975)

The above impairment of the initiating signal of glucose probably corresponds to a decrease in the sensitivity of the islet for the hexose since both in subjects with low insulin response and in some maturity-onset diabetics (Cerasi et al., 1972, 1973 a) and also in the Acomys (Gutzeit et al. 1974 b), the maximal response of the pancreas to glucose may be normal, but the threshold glucose concentrations needed to elicit release of insulin are higher than in controls (Fig. 14). Thus the glucose-insulin dose relationship is displaced towards the right of the control curve in a parallel manner both in diabetic man and in

nance the heritability of the compound variables was evaluated When the hypothesis of heritability alone was fitted to the data, the chi square values for the residual variance was *non-significant* In contrast when the hypothesis of the common environmental component alone was tested, highly significant residual chi squares were obtained These findings thus indicate that the insulin response to glucose is indeed *genetically regulated*. In this study the magnitude of the *genetic* component for some of the variables was as high as 0 72.

At this point a clarification has to be made Firstly in more recent studies the degree of concordance *regarding glucose tolerance and* insulin response in monozygotic twins has been *found* to be variable (Pyke and Taylor 1967 Pyke et al 1970 Tattersal and Pyke 1972) The concordance was very high in the pairs in which the disease was diagnosed after the age of 40 In contrast only 50 % of *the pairs with juvenile diabetes were concordant* Furthermore in a recent report from *the Joslin Laboratory* (Gottlieb et al *1974*) the regression line correlating plasma insulin to blood glucose over the five hours of an oral glucose challenge was found to be steeper in monozygotic twins of diabetics than in controls *However the insulin – glucose ratio* early (15 min) during the load was smaller in *the twins Thus the insulin secretion may not* be reduced in twins of diabetic patients at later times during stimulations (see below) and furthermore this twin material may be less homogenous as to its true prediabetic nature than was earlier believed. These findings are in keeping with the idea that in *juvenile diabetes non-genetic factors* are of great significance for the development of the disease (see also p *121*)

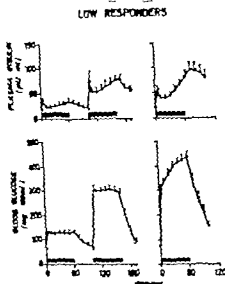
When glucose infusions were performed on a large group of healthy adults with normal glucose tolerance the majority showed the expected prompt and marked increase in plasma insulin. However in 15 to 20 % of the group the insulin response was similar to that found in diabetic and prediabetic subjects (fig 11) (Cerasi and Luft 1967 a) Since a *qualitatively and quantitatively identical impairment* in insulin response was found in overt diabetics latent diabetics, and genetic prediabetics it was concluded that impairment in insulin release might be a conditio sine qua non *for the development of the disease* By analogy the healthy subjects with *the same decrease and delay in insulin response* to glucose were asumed to be prediabetics or more correctly subjects in whom the probability to develop diabetes in later life is greater than in subjects with normal insulin response. Recent observations from our group suggest that subjects with *low insulin response indeed are more susceptible* to develop glucose intolerance (see below)

Low insulin response and decreased glucose tolerance is observed also in a laboratory animal the semi-desert rodent Acomys cahirinus (spiny mouse) which as will be seen below serves as an excellent model for studying *the mechanisms responsible for the deficiency* of the insulin response in diabetes (Gutzeit et al *1974 a b*)

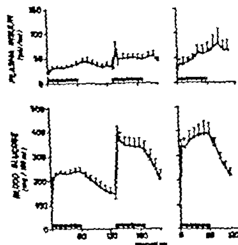
As mentioned above (p *121*) glucose exerts two distinct actions on the release of insulin initiation and potentiation. The role of these functions in the impairment of insulin secretion in diabetes will be discussed below

a Initiation of insulin release by glucose in the diabetic syndrome

It has often been said – also by ourselves – that the defective release of insulin concerns mainly the initial phase of the response However it can be shown in many patients *with mild maturity-onset diabetes* and in experimental models such as the spiny mouse *that the kinetics of insulin release* are not necessarily grossly modified but that the totality *of the response is reduced when certain* types of glycemic stimulations are applied Therefore *and in accordance with* our earlier statement in this review on the



A



B

Fig. 15
Enhancement of the insulin response to glucose infusion by pretreatment with glucose in subjects with decreased insulin responsiveness (A) and patients with maturity-onset diabetes (B). In (A) the glucose doses used were 10 mg/kg initially followed by 5 mg/kg/min from 0 to 60 min and 500 mg/kg + 20 mg/kg/min from 100 to 160 min (the right-hand panel shows control experiments corresponding to the second glucose infusion). In (B) 250 mg/kg + 5 mg/kg/min was given at 0-60 min and 500 mg/kg + 10 mg/kg/min at 130-190 min. The corresponding control experiment at the right-hand panel

Note in both instances the changes towards normality that occur in the blood glucose as well as plasma insulin curves when the challenge was preceded by a glucose infusion. (From Cerasi 1975 d).

The type of the potentiation induced at least in low insulin responders in whom several experiments could be performed is similar to that found in the controls, i.e. a multiplicative one. The difference between the results in these two groups resides in the magnitude of the effect, which seems more important in the low insulin responders. This is clearly apparent when the sensitivity of the diabetic and prediabetic islet for the potentiating action of glucose is considered. Our studies in normal subject demonstrated that the sensitivity of the potentiation for glucose was low compared to the sensitivity of the islet for the insulin releasing effect of the hexose. Paradoxically in low insulin responders and in mild diabetics the islet seem to have higher sensitivity for the potentiating effect of glucose. In Fig. 16, the dose response relationship between the mean blood glucose level of the preinfusion period and the per cent enhancement of the insulinogenic index is compared with the results in the controls. It is clearly seen that low insulin responders seem to be more sensitive than the controls, since the curve is left-shifted and demonstrates higher per cent enhancements. As to the diabetics, in whom only a limited study was possible, they seem to respond like the normal subjects. These findings are striking if one remembers that the dose-response curves for the acute insulin releasing action of glucose are shifted to the right in these subjects (Fig. 14). Thus, the sensitivity of the pancreas for glucose – the

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Although it may be hazardous to draw firm conclusions from studies in man in whom, e g a maximal insulin response practically never can be achieved we do believe that the basic defect in the mild forms or initial stages of diabetes manifests itself as decreased sensitivity of the recognition of the β-cell for glucose (increase in K_m) Low insulin response has been demonstrated already in early childhood (Cerasi and Luft 1970 b) It is not known whether this type of response is inherited or appears later during postnatal life In this connection it should be reminded that during fetal life and immediately following birth the normal pancreas shows a similar insensitivity to glucose (see Asplund 1972) which also here is accompanied by a deficient cyclic AMP response to the hexose (Grill et al 1975) Within a few days after birth a functional maturation of the β-cells appears with restoration of the glucose effects The possibility remains that low insulin response in man reflects an incapacity of the β-cells to undergo the normal maturation process

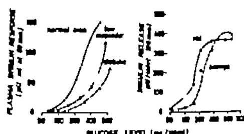
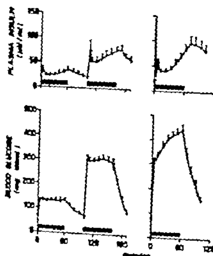


Fig 14
Dose-response relationship of glucose-induced late insulin response in the various developmental stages of human diabetes left data from Cerasi et al (1972) and right in islets of Acomys cahirinus incubated during 120 min, data from Gutzeit et al (1974 b).

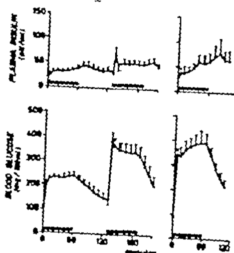
Experiments in man confirm this postulation (Cerasi 1975 d) In the majority of low insulin responders and in a certain number of patients with maturity-onset diabetes, pretreatment with glucose strongly enhances the insulin response to a subsequent glucose challenge (Fig 15 a) After priming with glucose in many low insulin responders the blood glucose and plasma insulin responses to glucose infusion were not distinguishable from those in high insulin responders and even the mean responses in a larger heterogenous group of subjects fell within the range of the non-primed normal ones The same cannot be claimed for the mean values in the diabetic subjects in whom the responses, even after priming, are much inferior to normal In these the glucose preinfusion seems to convert the insulin response from the diabetic one to that seen in low insulin responders (15 b) However it is striking that, at least in some patients although they may present the typical depressed and delayed insulin response to glucose this response may be totally transformed into a normal one simply be creating a state of potentiation in the islets by the glucose pretreatment These results confirm our earlier statement (by using adrenaline) that a normal insulin response is not a prerequisite for the generation of potentiation by glucose

b *Potentiation of insulin release by glucose in the diabetic syndrome*

There is no evidence in the literature indicating that the glucose utilization of islets from animals deficient in their insulin response should be grossly modified Recent experiments with islets of Acomys confirm that under conditions where the islets respond to glucose with decreased insulin output a normal rate of glucose utilization does occur (Cuendet et al cited in Cerasi 1975 e) Therefore since we postulated previously in this review that the potentiating action of glucose is generated by the utilization of the hexose in the β-cell (Fig. 10) potentiation may be expected to be intact in the diabetic islet.



A



B

Fig. 15
Enhancement of the insulin response to glucose infusion by pretreatment with glucose in subjects with decreased insulin responsiveness (A) and patients with maturity-onset diabetes (B). In (A) the glucose doses used were 10 mg/kg initially followed by 5 mg/kg/min from 0 to 60 min and 500 mg/kg + 20 mg/kg/min from 100 to 160 min (the right-hand panel shows control experiments corresponding to the second glucose infusion). In (B) 250 mg/kg + 5 mg/kg/min was given at 0-60 min and 500 mg/kg + 10 mg/kg/min at 130-190 min. The corresponding control experiments at the right-hand panel.

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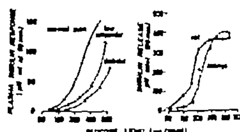


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Development of glucose intolerance

Although it has been stated previously in this review that low insulin response occurs in about 15-20 per cent of the normal population, it must be emphasized that there is no clear border between low and high responders. The insulin response to glucose infusion shows a continuous distribution from the lowest to the highest ones without the appearance of sub-populations. The term low insulin responder applies to the left tail of the distribution curve the limit necessarily being an arbitrary one.

Our working hypothesis has been that the risk for developing glucose intolerance is inversely correlated to the magnitude of the insulin response For this reason, the glucose tolerance of groups of high and low insulin responders has been controlled over a period of several years. Furthermore some of the factors, apart from insulin that participate in the regulation of glucose homeostasis have been investigated.

1 Follow-up of low insulin responders

Since manifest diabetes is present in only % of the population no more than each tenth low insulin responder would be xpected to develop diabetes sometimes during life. Consequently substantial number of such individuals would have to be observed for decades until definite answer can be given to the question whether prediabetes as defined by us is prerequisite for the development of diabetes

We have ecently completed the first follow-up study of our prediabetics regarding the incidence of glucose intolerance defined as k value less than 1 0 (intravenous glucose tolerance test, IVGTT according to Ikkos and Luft 1957) Our groups comprised 94 controls nd 40 prediabetics, all non-obese, observed for a mean period of about four years (Cerasi and Luft 1974) The IVGTT was repeated at least once in all subjects and 3-7 k values were obtained in each of 78 high responders and 1 3 values in .5 low responders Table 1 shows that out of the 259 tests performed after the initial one in the high responders six (2 3 %) fell within the diabetic range The corresponding finding in the low responders was 21 out of 116 (18 1 %) In the high responders abnormal glucose tolerance was observed on one single occasion in three subjects and three times in only one individual I the low responders single low k-values were noted in nine subjects while diabetic values occurred 7 times in another three It should be added that in the majority of subjects – even in those with several decreased k-values – glucose intolerance was transient In only two subjects both belonging to the low responding group glucose intolerance has become permanent.

In this connection it should be emphasized that, in our prediabetics the k value at the beginning of the observation period was significantly lower than in the control group 1 46 ± 0 05 and 1 94 ± 0 07 respectively (p <0.01) This indicates that the control of glucose homeostasis is not as efficient in prediabetics as in the control population. There fore it is not surprising if in the low insulin responders the control of glucose homeostasis occasionally became still less efficient and glucose intolerance ensued.

In all, these data suggest that low insulin responders do indeed run a greater risk of developing latent diabetes than persons with normal insulin output, even over relatively short observation periods It is of interest in this connection to note that those four of the high insulin responders who later developed glucose intolerance demonstrated insulin re

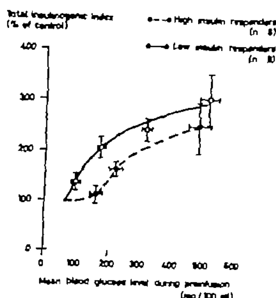


Fig 16
Dose-dependency of the potentiating action of glucose on glucose-induced insulin release The insulin response to a fixed-dose glucose infusion was measured with and without prior glucose infusion at varying doses Total insulinogenic index was calculated as the total plasma insulin areas of the glucose infusion divided by the blood glucose area The index of the control experiment is taken as 100 % The abscissa shows the mean blood glucose level achieved during the 100 min that preceded the second glucose infusion test (From Cerasi 1975 f)

initiator of release – is decreased whereas the sensitivity for glucose – the potentiator – is at least normal in the early stages of the diabetic syndrome

The significance of the apparent increase in sensitivity for potentiation that was observed in low insulin responders is difficult to assess It may be the consequence of the chronic (albeit minimal) stimulus for potentiation provided by the somewhat higher blood glucose levels observed after glucose (and probably after meals) in these subjects (Cerasi and Luft 1974) This could function as a compensatory mechanism, thus limiting the deficiency in insulin response. Whether there is actually loss of this increased sensitivity in diabetic patients is impossible to establish on the basis of the limited information available at present

7 Conclusions

The role assigned by nature to the pancreatic β-cell during evolution – that of controlling the glucose homeostasis of the organism has led to the appearance of a dualism in the cell regarding glucose While utilizing glucose for a number of specialized and non-specialized needs as all other cells, the β-cell has developed a cybernetic system for the tight coupling of the extracellular information to the discharge of insulin Our present understanding of this dual aspect of the β-cell is rudimentary but of utmost importance for the clarification of the physiologic behaviour of the islets and the modifications that appear during the development of maturity-onset diabetes mellitus. Thus the initial defect in the diabetic β-cell seems to consist in a very limited modification of a specific step in the recognition of hyperglycemia as the acute trigger of the processes that lead to release of insulin (mutation of the glucoreceptor?) In spite of its limited character (decrease of sensitivity) the strategic location of this defect may cause important delays in the feed-back loops that regulate the glucose homeostasis of the organism and therefore be sufficient to impair the glucose tolerance The findings presented here justify our previous suggestion of regarding diabetes mellitus as a disorder of cellular information transmission (Cerasi and Luft 1970) It may be expected that once the molecular basis for this modification clarified our means of treating and preventing diabetes mellitus will change radically

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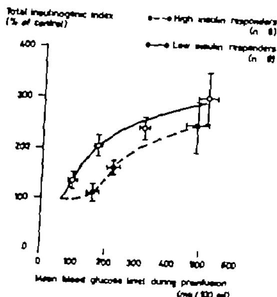


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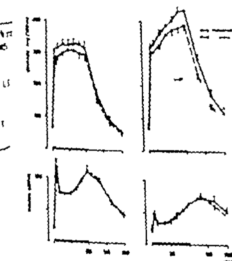


Fig 18
Insulin response to glucose infusion in 33 subjects with high (A) and 27 with low (B) insulin response at the beginning of the study and 3.6 ± 0.4 and 2.5 ± 0.3 years later (From Cerasi & Luft 1974)

tion of the glucose tolerance will be considered in the following.

2. Factors precipitating glucose intolerance in prediabetics

It is well known that latent and manifest diabetes often make their debut in connection with other disorders such as obesity acromegaly Cushing syndrome etc and also in connection with pregnancy Three such precipitating conditions will be discussed obesity acromegaly and pregnancy They all share the occurrence of peripheral resistance to insulin

a Obesity

Diabetes is much more common in obese people than in the general population It is also well established that obesity is accompanied by an increased insulin response to hyperglycemia (Karam et al. 1963 1965 Beck et al. 1964 Kreisberg et al 1967 Luft et al 1968) From this it can be concluded that obese people must be less sensitive to insulin As a matter of fact, it has been demonstrated that in man obesity renders adipose tissue resistant to insulin (Salans et al 1968). Muscle (Felig et al. 1969) and liver (Cahill, 1971) may also participate in the generalized hyposensitivity to insulin in obesity Recent studies in the obese-hyperglycemic (ob/ob) mouse by Kahn et al (1973) suggest that the primary defect in insulin resistant organs (adipose tissue and liver) is a decrease in the number of receptor sites for insulin in the cell membranes of these tissues.

From the above follows that obese people must secrete increased amounts of insulin in order to keep the glucose tolerance normal. In prediabetics the β-cells would not be capable to adequately enhance insulin secretion in order to compensate for insulin resistance This would then lead to glucose intolerance

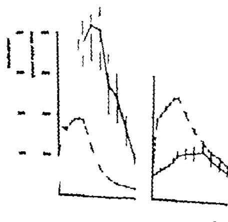


Fig 19
Glucose infusion test in A group of obese non-diabetic subjects and B group of obese diabetic subjects Open circles and broken lines denote the mean blood glucose levels filled circle and solid lines the mean plasma insulin levels The vertical bars stand for ± S.E.M (From Luft et al 1968)

Table I
Results of repeated IVGTT in high and low insulin responders

Period of follow-up years	No. of repeated IVGTT	No of k-values below 1 0	No of subjects with k <1 0
I *High insulin responders* 4 4 ± 0.2 (range 1 0-9 8)	(n=94) 259	6 (2.3 %)	4 (4.3 %)
II *Low insulin responders* 3.5 ± 0 4 (range 0 9-9 0)	(n=40) 116	21 (18.1 %)	12 (30 %)

sponses which were lower than for the rest of the group It may be questioned whether these subjects really belong to the group of high responders or formed an intermediate group between high and low responders Again it is essential to remember that the criteria used for delimiting low from high insulin responders are only arbitrary (Cerasi and Luft 1967 a)

One obvious question is whether the glucose intolerance when it appears in prediabetics is due to a further decrease in the insulin secretory capacity Our studies on the above groups of subjects do not support such an interpretation As a matter of fact insulin release appeared *surprisingly stable in a* group of nine prediabetics who developed glucose intolerance (Fig. 17) – as well as in the whole material of controls and prediabetics submitted to a glucose infusion test on two occasions with an interval of 2 5 ± 0 3 years (Fig 18)

In this connection we wish to refer to the dose response curves for the glucose-insulin relationship presented in Fig. 14 These clearly demonstrate that the decrease in the β-cell sensitivity for glucose is more pronounced in subjects *with mild diabetes than* in prediabetics Therefore it cannot be excluded that one factor leading to the precipitation of glucose intolerance in prediabetics may be the progressive deterioration of the insulin secreting *capacity of the* β-*cells* Other factors contributing to the deteriora-



Fig 17
Insulin response to glucose infusion in 9 low insulin responders at the time of normal and of decreased k values (From Cerasi & Luft 1974)

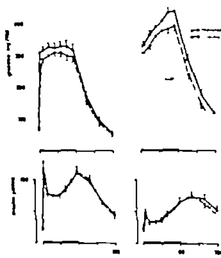


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Fig 19 demonstrates clearly the different patterns of insulin release in obese subjects with normal and decreased glucose tolerance In the former group insulin response to a glucose challenge was excessive reaching a peak value around 400 μU/ml In the latter group the insulin curve was of the type seen in prediabetics and diabetics and the peak value reached was only about 110 μU/ml

Information is still lacking regarding β-cell function in low insulin responders before and after the development of obesity In spite of this the above findings are consistent with the view that subjects with low insulin response are less fitted to cope with metabolic situations that require major enhancement of insulin production

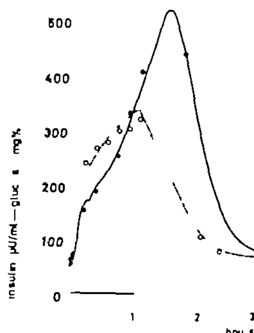


Fig 20 A
Glucose infusion test in acromegaly A patients with normal glucose tolerance and moderate to high degree of activity of acromegaly B patients with normal glucose tolerance and low activity of the disease C patients with glucose intolerance irregard of the activity of acromegaly Filled circles denote mean plasma insulin open circles mean blood glucose values Solid curves denote insulin levels broken curves blood glucose levels both obtained by analogue computation Glucose was infused between 0-1 hours (From Luft et al 1967)

b Acromegaly

It is well known that diabetes is more common in acromegalic subjects than in the general population This has been ascribed to the peripheral resistance to insulin induced by the overproduction of growth hormone (GH) in this disease (Ikkos and Luft 1962) In spite of this diabetogenic action of GH the incidence of glucose intolerance in acromegalics – at least in our groups of about 150 such patients – was not higher than around 25 % This is explained by the enhanced insulin secretion induced by the high levels of GH This comprises basal (Ehrlich and Randle 1961) as well as stimulated insulin release (Cerasi and Luft 1963 Luft et al 1967)

An illustration of the increased insulin secretion in acromegaly is presented in Fig. 20 (A and B) the more active the acromegalic state the more enhanced was the capacity of the β-cells of the pancreas to secrete insulin Successful treatment of the acromegaly in these subjects by surgical hypophysectomy implantation of radioactive ytrium in the sella turcica or administration of large doses of oestrogens was followed by a marked regression of the hyperresponsiveness of the pancreatic β-cells to glucose

As demonstrated in Fig 20 C in acromegalic patients with glucose intolerance or mild diabetes the insulin response to glucose was sluggish and decreased as in diabetes. Successful treatment of this group of subjects was accompanied by normalization of the glucose tolerance while the insulin response remained of the diabetic type

These results may indicate that the acromegalic subjects with glucose intolerance originally were prediabetics and that diabetes developed because of their inability to increase substantially the insulin production during

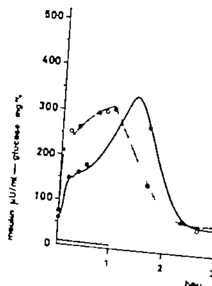


Fig 20 B

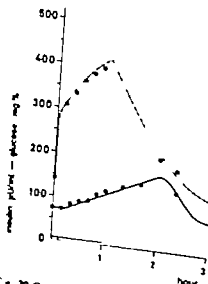


Fig 20 C

GH hypersecretion. Therefore they were unable to cope with the increased demand for insulin in order to overcome the peripheral insulin resitance This statement is supported by experiments showing that GH in dose of 0 3 mg/kg/day during four days significantly enhanced insulin secretion in normal subjects whereas no such effect was noted in prediabetics (Luft et al 1969). In this connection, it is also noteworthy that the incidence of diabetes in acromegaly (about 25 %) approximately coincides with the incidence of prediabetes (about 20 %) in our normal population.

c Pregnancy

It s well known that pregnancy can provoke diabetes mellitus. Furthermore women who later become diabetics can experience an altered outcome of pregnancy with increased perinatal mortality malformations and large-for-date babies long before the clinical manifestation of the disease (Hadden and Harley 1967 Lunell and Persson, 1972).

These findings may be partially ascribed to the increased peripheral resistance to insulin which occurs during pregnancy most probably due to overproduction of chorionic somatotropin (hCS) (Beck and Daughaday 1967 Samaan et al. 1968 Kalkhoff et al 1970) However in women developing diabetes during pregnancy some other diabetogenic factor must be present since pregnant healthy women despite the increased peripheral resistance maintain a normal glucose tolerance This additional factor – in analogy to the situation in obesity and acromegaly – could be a decreased capacity of the pancreatic β-cells to respond normally to insulinogenic stimuli. As a matter of fact it has been well documented that the insulin response to glucose is increased during pregnancy (Spellacy and Goetz 1963 Bleicher et al. 1964 Spellacy et al 1965 a, b). This may be due to the increased production of the two pregnancy hormones hCS and progesterone. The former has been shown to stimulate insulin

Fig. 19 demonstrates clearly the different pat terns of insulin release in obese subjects with normal and decreased glucose tolerance In the former group insulin response to a glucose challenge was excessive reaching a peak value around 400 μU/ml In the latter group the insulin curve was of the type seen in prediabetics and diabetics and the peak value reached was only about 110 μU/ml

Information is still lacking regarding β-cell function in low insulin responders before and after the development of obesity In spite of this the above findings are consistent with the view that subjects with low insulin re sponse are less fitted to cope with metabolic situations that require major enhancement of insulin production

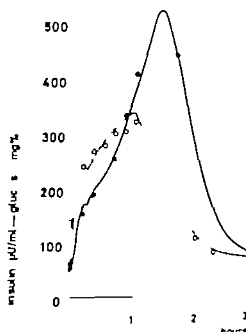


Fig 20 A
Glucose infusion test in acromegaly A patients with normal glucose tolerance and moderate to high degree of activity of acromegaly B patients with normal glucose tolerance and low activity of the disease C patients with glucose intolerance irregard of the activity of acromegaly Filled circles denote mean plasma insulin open circles mean blood glucose values Solid curves denote insulin levels broken curves blood glucose levels both obtained by analogue computation Glucose was infused between 0-1 hours (From Luft et al 1967)

b Acromegaly

It is well known that diabetes is more common in acromegalic subjects than in the general population This has been ascribed to the peripheral resistance to insulin induced by the overproduction of growth hormone (GH) in this disease (Ikkos and Luft 1962) In spite of this diabetogenic action of GH the incidence of glucose intolerance in acromegalics – at least in our groups of about 150 such patients – was not higher than around 25 % This is explained by the enhanced insulin secretion induced by the high levels of GH This comprises basal (Ehrlich and Randle 1961) as well as stimulated insulin release (Cerasi and Luft 1963 Luft et al 1967)

An illustration of the increased insulin secretion in acromegaly is presented in Fig 20 (A and B) the more active the acromegalic state the more enhanced was the capacity of the β-cells of the pancreas to secrete insulin Successful treatment of the acromegaly in these subjects by surgical hypophysectomy implantation of radioactive yttrium in the sella turcica or administration of large doses of oestrogens was followed by a marked regression of the hyperresponsiveness of the pancreatic β-cells to glucose

As demonstrated in Fig. 20 C in acromegalic patients with glucose intolerance or mild diabetes the insulin response to glucose was sluggish and decreased as in diabetes. Successful treatment of this group of subjects was accompanied by normalization of the glucose tolerance while the insulin response remained of the diabetic type

These results may indicate that the acromegalic subjects with glucose intolerance originally were prediabetics and that diabetes developed because of their inability to increase substantially the insulin production during

GH hypersecretion Therefore they were unable to cope with the increased demand for insulin in order to overcome the peripheral insulin resitance This statement is supported by experiments showing that GH in a dose of 0.3 mg/kg/day during four days significantly enhanced insulin secretion in normal subjects, whereas no such effect was noted in prediabetics (Luft et al 1969) In this connection, it is also noteworthy that the incidence of diabetes in acromegaly (about 25 %) approximately coincides with the incidence of prediabetes (about 20 %) in our normal population.

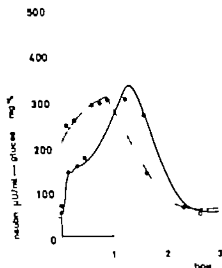


Fig 20 B

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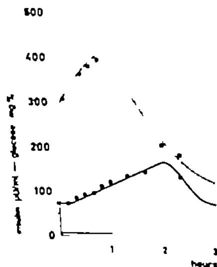


Fig 20 C

release and synthesis in vitro (Malaisse et al 1969 Martín and Friesen 1969) and progesterone to increase insulin response to glucose in vivo (Beck 1969 Kalkhoff et al 1970)

In addition in a recent study on a limited number of pregnant prediabetic women and controls we were able to demonstrate (Edström et al 1974 a, 1975) that only in the prediabetic women – although they did not develope diabetes – did glucose tolerance decrease significantly during the last trimester The insulin response to glucose infusion increased markedly throughout the pregnancy in all women but was significantly less in those demonstrating a low insulin response in the non-pregnant state The non-pregnant prediabetic women demonstrated a significantly higher sensitivity to endogenous insulin than the controls during pregnancy this sensitivity decreased in both groups reaching the same level in the third trimester These changes could not be ascribed to differences between the two groups regarding plasma levels of hCS and the excretion of oestriol In addition the infants of the prediabetic mothers demonstrated a significantly higher mean glucose tolerance during the first day of life than those of the control women approaching the values found in infants of diabetic mothers

In summary pregnancy is accompanied by insulin resistance which is compensated by enhanced insulin secretion In prediabetic women during pregnancy this secretion remains low in comparison to normal women *In addition their originally high sensitivity to* endogenous insulin decreases These two facts bring these women closer to a diabetic state in which insulin production may become insufficient to cover the increased demand

d Conclusions

In all three conditions discussed above – obesity acromegaly and pregnancy – glucose intolerance *is much more common than in the* general population A common denominator for all of them is increased peripheral resistance to insulin In normal subjects this resistance is compensated by hyperresponsiveness of the pancreatic β-cells to insulinogenic stimuli As a net result glucose tolerance is kept normal This is not the case in low insulin responders in whom the capacity to increase the insulin response is diminished. Therefore in these subjects the balance between insulin sensitivity and insulin release is deranged leading to impairment of the glucose tolerance.

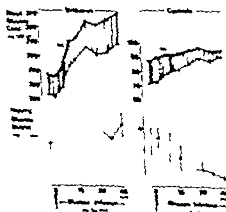
3 The role of the liver in counteracting glucose intolerance in low insulin responders

Since in prediabetic subjects – or low insulin responders – normal glucose tolerance is maintained in spite of diminished insulin secretion we have suggested that some compensatory mechanism might operate preventing the appearance of glucose intolerance (Cerasi and Luft 1970 a) Indeed it was demonstrated that endogenous (Cerasi and Luft 1967 c) as well as exogenous (Martin et al 1968 Cerasi and Luft 1969 b) insulin is more effective in lowering blood glucose in prediabetic subjects On the basis of studies on the conversion of [14]C pyruvate to [14]C-glucose it was suggested that the site of the increased insulin sensitivity in prediabetes might be the liver (Cerasi and Luft 1967 c Shreeve et al. 1970)

Early studies in non-ketotic diabetic patients had demonstrated that the rate of splanchnic *glucose production was not altered in* diabetes (Myers 1950 Bearn et al 1951 Shreeve et al. 1964 Manougian et al 1964) This was confirmed recently by our group (Wahren et al 1972) However in our studies the splanchnic uptake of alanine and other glycogenic amino acids was 1 5-2 times greater in the diabetics while lactate and pyruvate uptake was increased by 65-115 % Splanchnic uptake of these glucose precursors could ac*count for 32 % of hepatic glucose output in* the diabetics as compared to 20 % in the

controls. This increase in precursor uptake was the consequence of a 2 3-fold increment in fractional extraction of these substrates. Administration of glucose in a concentration as low as 2 mg/kg/min resulted in an 80 % reduction in splanchnic glucose output in the controls, but failed to inhibit hepatic glucose release in the diabetics despite a twofold greater increment in arterial glucose levels. This is illustrated in Fig. 21

It could be concluded that, in non-ketotic diabetics total splanchnic output of glucose is comparable to that of controls but the relative contribution of gluconeogenesis may be increased by more than 50 %. Furthermore, accelerated splanchnic uptake of glucose precursors is a consequence of increased hepatic extraction of available substrates rather than a result of augmented substrate supply. The failure of hyperglycemia to inhibit hepatic glucose output suggests that the exquisite sensitivity of the liver to the infusion of glucose in normal man is a consequence of glucose induced insulin secretion.



F g 21
Mean arterial (A) and hepatic venous (HV) glucose concentrations and splanchnic glucose production in the basal state and during infusion of glucose at rate of 2 mg/kg per min for 45 min in diabetic and seven control subjects. Mean values ± SE. (From Wahren et al. 1972)

With this knowledge as background studies were undertaken aiming at an explanation for the normal glucose tolerance in low insulin responders (Cerasi et al. 1973 b; Wahren et al. 1973). It was found that, in the basal state the splanchnic glucose output was 45 % lower than in the control group in spite of the significantly reduced basal insulin levels (Table 2). The splanchnic extraction of gluconeogenic substrates was similar to that of the controls (Table 2) and, therefore their contribution to splanchnic production of glucose of the same magnitude in both groups. Consequently the diminished glucose output in the prediabetics is probably due to a decrease in hepatic glycogenolysis. Furthermore, infusion of 2 mg/kg/min of glucose was accompanied by a significantly greater decrease in hepatic glucose output than in the controls in spite of reduced insulin levels (Fig. 22)

Thus it seems that in prediabetics, glucose tolerance is kept normal by decreased splanchnic production of glucose in the basal state as well as during hyperglycemia.

The question arises whether a prediabetic secretes smaller amounts of insulin because of increased insulin sensitivity and hence has a reduced need for the hormone. It should then be pointed out that low insulin responders have a slightly higher blood glucose level and significantly lower k values (see above). They also reach higher blood glucose levels during glucose infusion and their peripheral utilization of glucose in the basal state as well as during hyperglycemia is reduced. Thus these subjects have a diminished rate of glucose turnover a finding which is not compatible with an overall increased insulin sensitivity in the body

The mechanism of increased sensitivity of the liver to insulin in prediabetes is unknown. It has been demonstrated that insulin binds to

release and synthesis in vitro (Malaisse et al 1969 Martin and Friesen 1969) and progesterone to increase insulin response to glucose in vivo (Beck 1969 Kalkhoff et al 1970)

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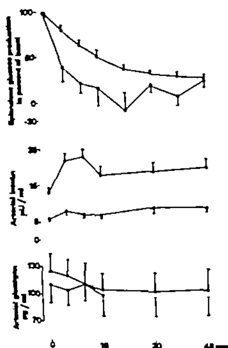


Fig. 22
Arterial conc trations of gluc gon and insulin and perc ntage change of planchnic gluc se production during the infusion of 2 mg glucose/kg/min for 45 min. Open circles control filled circl low insuli esponders ertical bars S.E. of th mean. Basal pl nchnic gluco production i given as 100 per ent (From Cerasi et al 1973 b)

tion of the sensitivity to endogenous insulin – especially since the diabetic patient may have high plasma glucagon levels it is possible that at a stage when glucose tolerance is impaired low insulin responders secondarily develop a certain extent of insulin insensitivity which accelerates the appearance of manifest diabetes.

Under any circumstance it seems to us unlikely that moderate insulin resistance per se may ever induce diabetic state. The marked insulin resistance which accompanies obesity pregnancy acromegaly etc. is counterbalanced by the pronounced adaptability of the normal islets to the needs of the body (see above). In animal models, like the ob/ob mouse where mild diabetes does occur the plasma insulin levels at the basal state and after stimulation are extremely elevated compared to the lean controls. A comparable situation does not exist in mild maturity-onset diabetes. Therefore if some degree of insulin resistance is present in early diabetics it probably acts as a secondary additive factor rather than a primary one

It may be added that some authors, using other techniques, could not demonstrate insulin resistance in mild diabetes (Thorell et al 1975)

Table 2
Balance of glucose and glucogenic substrates across the planchnic vascular bed in the basal state in low insulin responders and controls

	Low insulin responders	Controls
Glucose production[a]	0.680	1.230
Uptake of		
(1) Lactate[b]	0.120	0.110
(2) Pyruvate[b]	0.011	0.011
(3) Amino acids[b]	0.066	0.056
(4) Glycerol[b]	0.024	0.021
Sum of (1)-(4)	0.221	0.198
Glucose production not accountable for substrate uptake	0.459	1.032

Data presented as mmoles/min
[b] Expressed as glucose equivalents in mmoles/min
Sum of splanchnic uptake of amino acids for which a statistically significant uptake was found.

specific receptors in liver membranes (Freychet et al 1971) and that this binding might be modified under different experimental conditions In mice with the obese-hyperglycemic syndrome there was a 60-80 per cent decrease in insulin receptors in isolated hepatocytes and in plasma membranes of both liver and fat (Kahn et al 1973 b) Acute and chronic dietary restriction in these animals ameliorated the insulin resistance and was accompanied by an increase in insulin receptor sites towards normal In animals treated with insulin during weight reduction an increase in insulin binding sites was prevented. Thus it appears that a major factor modulating the insulin receptor sites in obese-hyperglycemic animals is hyperinsulinemia itself Also aging seems to decrease the insulin binding capacity of hepatic plasma membranes for insulin (Freeman et al 1973)

If one extrapolates from these animal models to man it may be speculated that in prediabetics the low insulin response is compensated by an increased number of receptor sites for insulin in the liver This compensatory mechanism would keep gluconeogenesis within normal limits but allow glycogenolysis to be markedly suppressed. This idea is supported by the recent finding in dogs that glycogenolysis is far more sensitive to small changes in insulin concentration than is gluconeogenesis and that relatively large amounts of insulin are required to suppress gluconeogenesis from alanine (Liljenquist et al 1974). The loss of this compensatory mechanism ought to lead to increased gluconeogenesis and, hence to the transition from prediabetes to latent diabetes How this comes about is not known As suggested by Freeman et al. (above) in animal experiments aging may be of importance in this connection

Contrasting with the data and the hypothesis presented in this chapter are the studies of the group of Reaven (Shen et al 1970 Ginsberg et al 1974) These authors demonstrated that the steady state blood glucose level achieved by administration of glucose and insulin in non-obese patients with chemical diabetes was much higher than in normal subjects when similar plasma insulin levels were induced indicating that the former group demonstrated resistance to insulin action Although it is not clear to us whether the experimental protocol used by the authors (simultaneous infusion of epinephrine propranolol glucose and insulin) permits evalua

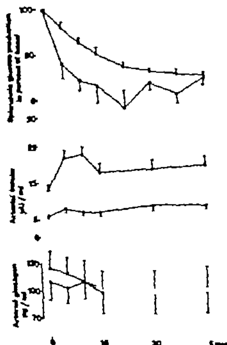


Fig. 22
Arterial concentration of glucagon and insulin and percent ge hange of splanchnic glucos production dur g th Inf sion of 2 mg glucos /kg/min for 45 min. Open circles ont ol filled circles low insuli espon- ders vertical bars S.E. of the mean. Basal splanchnic glucos production is given as 100 per ent (From Cerasi et al 1973 b)

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Relationship between insulin secretion and development of vascular disease

It has long been a matter of common knowledge that conditions due to arteriosclerosis occur earlier and are more extensive in diabetics than in non-diabetics. According to Entmacher et al (1964) all vascular disorders registered were at least twice as common in diabetic as in non-diabetic males and females and in toto were 10-20 times more common in younger diabetics than in non-diabetics of this age group.
Similarly, diabetics are *overrepresented* among subjects with myocardial infarction (Levine and Brown 1929, Clowson and Bell 1949, Eckerström 1951, Lindén 1952, Wahlberg 1966). Furthermore the long term prognosis of diabetic survivors from myocardial infarction was found to be less favourable than that of non-diabetic ones (Biörck et al. 1958, Sievers 1964, Partamian and Bradley 1965).

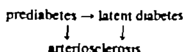
All these data refer to findings in subjects with manifest diabetes. The obvious question to be asked is whether a similar correlation could be found between the earlier phases of *the diabetic syndrome* – prediabetes and latent diabetes – and arteriosclerosis. Along this line are a series of studies published since 1934 claiming that a decrease in oral glucose tolerance (latent diabetes) occurred much more often in subjects with arteriosclerosis than in the normal population. The incidence of abnormal tests ranged from 35 to 85 %. The groups studied by most authors comprised survivors from myocardial infarction (for review, see Luft et al. 1973).

Only few studies have been reported where the intravenous glucose tolerance was measured in patients with arteriosclerosis. Even here the incidence of abnormal tests was common, ranging somewhere between 25 and 65 % (Luft et al., 1973).

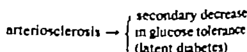
Of special interest are the studies by Wahlberg (1966) and Heinle et al. (1969) which included appropriate control groups and a satisfactory number of subjects. They found almost the same incidence of diabetic and borderline tolerances in subjects with myocardial infarction, angina pectoris and intermittent claudication.

Accepting the fact that latent diabetes is common in arteriosclerotic vascular disease, we may raise the question whether this decrease in glucose tolerance is a manifestation of genetic diabetes mellitus, or if it is secondary to the vascular and metabolic changes that accompany arteriosclerosis. This question is a crucial one within the frame of preventive cardiology. If we include the prediabetic state into the picture we may illustrate the two alternatives in the following way:

a. in subjects *with* genetic diabetes



b. in subjects *without* genetic diabetes



Against this background we shall, in the following, discuss and reevaluate available data in the literature concerning insulin response to glucose stimulation in arteriosclerotic vascular disease. The data obtained with oral glucose tolerance tests are *presented* in Table 3. All of the authors found enhanced insulin release in their groups of patients, although

Table 3
Oral glucose loading and insulin release in patients with arteriosclerosis

Author	Disease		Time after infarction	Abnormal OGTT in %	Insulin release	p
Peters and Hales (1965)	Myocardial inf	7	6-17 months	Normal	Enhanced	p <0.05
Nikkilä et al (1965)	Myocardial inf	21	4 weeks -9 months	Decreased in 29 %	Enhanced in 33 % Decreased in 20 %	
Tzagournis et al. (1967)	Myocardial inf Angina pectoris	25	>1 month	Decreased in 40 %	Enhanced in 76 %	p <0.05
Kashyap et al. (1970)	Myocardial inf. Angina pectoris	18	3 weeks -4 months	Decreased in 72 %	Enhanced	p <0.02 p <0.005
Sloan et al (1970)	Arteriosclerotic vascular disease	51	>1 year	Decreased in 45 %	Enhanced	p <0.05 p <0.001
Malherbe et al. (1971)	Myocardial inf	70	>3 months	Decreased in 100 %	Enhanced	p <0.05 p <0.01

the oral glucose tolerance was decreased in most instances. This might be interpreted as showing that one of the essential defects in ischemic heart disease is peripheral resistance to insulin accompanied by secondary hyperinsulinemia. This finding even promoted speculation on the role of hyperinsulinemia per se in the pathogenesis of arteriosclerosis.

However the data in Table 3 should be reconsidered in the light of the above discussion on insulin secretion. In all studies the oral glucose tolerance was decreased in a considerable number of subjects. In reality the implication of this is higher than normal glucose stimulus resulting in a higher than normal insulin release. Therefore since no calculation was made of the glucose-insulin relationship no definite conclusion can be drawn regarding the type of insulin release. We have recently studied the dose-response relationship between the plasma glucose level and insulin release after an oral glucose load (Cerasi et al. 1973 b). A slight increase in glucose concentration resulted in a prominent enhancement of the insulin level indicating that insulin responses to oral glucose cannot be compared between different groups unless the blood glucose curves are identical.

The insulin release on intravenous glucose administration revealed results opposite to those above indicating decreased insulin response in coronary heart disease. Thus Nikkilä et al (1965) measured insulin release on i.v. glucose loading in 21 non-obese patients after myocardial infarction. A reduced glucose disappearance rate was found in four of these (19 %). Many of the patients with coronary heart disease had a low insulin response but on the whole, the difference between these and the controls was insignificant.

Recently Christiansen et al. (1968) (Table 4) and Boden (1971) (Table 5) demonstrated that the kinetics of insulin release on i.v. glucose injection in subjects with coronary heart disease and decreased intravenous glucose tolerance satisfied the criteria of Cerasi and Luft (1970 a) for diabetes, in this case latent diabetes. This was true for about 50 % of the patients of Christiansen et al. (1968) and 40 % of those of Boden (1971). Since no calculations were performed of the individual glucose-insulin relationships no statement can be made on the prevalence of prediabetes in the patient material. It has to be remembered that the ratio of prediabetics to latent diabetics has been estimated to about 4:1 in the population studied by Cerasi and Luft (1970 a).

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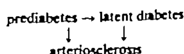
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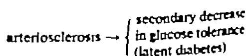
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the oral glucose tolerance was decreased in most instances. This might be interpreted as showing that one of the essential defects in ischemic heart disease is peripheral resistance to insulin accompanied by secondary hyperinsulinemia. This finding even promoted speculation on the role of *hyperinsulinemia* per se in the pathogenesis of arteriosclerosis.

*However the data in Table 3 should be re*considered in the light of the above discussion on insulin secretion. In all studies, the oral glucose tolerance was decreased in a considerable number of subjects. In reality the implication of this is a higher than normal glucose stimulus resulting in a higher than normal insulin release. Therefore, since no calculation was made of the glucose-insulin relationship no definite conclusion can be drawn regarding the type of insulin release. We have recently studied the dose-response relationship between the plasma glucose level and insulin release after an oral glucose load (Cerasi et al. 1973 b). A slight increase in glucose concentration resulted in a prominent enhancement of the insulin level indicating that insulin responses to oral glucose cannot be compared between different groups unless the blood glucose curves are identical.

The insulin release on intravenous glucose administration revealed results opposite to those above indicating decreased insulin response in coronary heart disease. Thus Nikkilä et al. (1965) measured insulin release on [illegible] glucose loading in 21 non-obese patients after myocardial infarction. A reduced glucose *disappearance rate was found in four of* these (19 %). Many of the patients with coronary heart disease had a low insulin response but on the whole, *the difference between* these and the controls was insignificant.

Recently Christiansen et al. (1968) (Table 4) and Boden (1971) (Table 5) demonstrated that the kinetics of insulin release on i.v. glucose injection in subjects with coronary heart disease and decreased intravenous glucose tolerance satisfied the criteria of Cerasi and Luft (1970 a) for diabetes in this case latent diabetes. This was *true for about 50 % of the patients of* Christiansen et al. (1968) and 40 % of those of Boden (1971). Since no calculations were performed of the individual glucose-insulin relationships no statement can be made on the prevalence of *prediabetes in* the patient material. It has to be remembered that the *ratio* of prediabetics to latent diabetics has been estimated to about 4 [illegible] 1 [illegible] in the population studied by Cerasi and Luft (1970 a).

Relationship between insulin secretion and development of vascular disease

It has long been a matter of common knowledge that conditions due to arteriosclerosis occur earlier and are more extensive in diabetics than in non-diabetics According to Entmacher et al (1964) all vascular disorders registered were at least twice as common in diabetic as in non-diabetic males and females and in toto were 10-20 times more common in younger diabetics than in non-diabetics of this age group
Similarly diabetics are overrepresented among subjects with myocardial infarction (Levine and Brown 1929 Clowson and Bell 1949 Eckerström 1951 Lindén 1952 Wahlberg 1966) Furthermore the long term prognosis of diabetic survivors from myocardial infarction was found to be less favourable than that of non-diabetic ones (Biörck et al 1958 Sieves 1964 Partamian and Bradley 1965)

All these data refer to findings in subjects with manifest diabetes The obvious question to be asked is whether a similar correlation could be found between the earlier phases of the diabetic syndrome – prediabetes and latent diabetes – and arteriosclerosis Along this line are a series of studies published since 1934 claiming that a decrease in oral glucose tolerance (latent diabetes) occurred much more often in subjects with arteriosclerosis than in the normal population. The incidence of abnormal tests ranged from 35 to 85 % The groups studied by most authors comprised survivors from myocardial infarction (for review see Luft et al 1973)

Only few studies have been reported where the intravenous glucose tolerance was measured in patients with arteriosclerosis Even here the incidence of abnormal tests was common ranging somewhere between 25 and 65 % (Luft et al 1973)

Of special interest are the studies by Wahlberg (1966) and Heinle et al (1969) which included appropriate control groups and a satisfactory number of subjects They found almost the same incidence of diabetic and *borderline tolerances in subjects with* myocardial infarction angina pectoris and intermittent claudication

Accepting the fact that latent diabetes is common in arteriosclerotic vascular disease we may raise the question whether this decrease in glucose tolerance is a manifestation of genetic diabetes mellitus or if it is secondary to the vascular and metabolic changes that accompany arteriosclerosis This question is a crucial one within the frame of preventive cardiology If we include the prediabetic state into the picture we may illustrate the two alternatives in the following way

a in subjects *with* genetic diabetes

prediabetes → latent diabetes
↓ ↓
arteriosclerosis

b in subjects *without* genetic diabetes

arteriosclerosis → { secondary decrease in glucose tolerance (latent diabetes)

Against this background we shall in the following, discuss and reevaluate available data in the literature concerning insulin response to glucose stimulation in arteriosclerotic vascular disease The data obtained with oral glucose tolerance tests are presented in Table 3 All of the authors found enhanced insulin release in their groups of patients although

Conclusions

Diabetes mellitus is generally regarded as a condition of heterogeneous origin. The present review deals exclusively with the pathogenesis of the adult type of the disease which is certainly the most common one. However the concepts presented here do not e clude that other factors may be of significance for the development of the disease in a propor tion of the diabetic population. Among such factors viral infections and islet autoimmunity may be cited

In diabetes mellitus of any severity the insulin response of the pancreas is deficient in relation to the needs of the organism. This is also the case in chemical or latent diabetes and in subject presumed to be potential diabetics The non-diabetic population demonstrates wide spectrum of insulin responses The insulin responses within the left hand tail of this distribution curve are similar to those found n diabetic subjects Therefore it was suggested that these normal' subject – with normal glucose tolerance – belonged to group with increased probability to develop diabetes Indeed 10 year follow-up study ha shown that subjects with low insulin re sponse had an 8-fold increased incidence of glucose intoler nce as compared to subject with high insulin response At present it is not known whether glucose intolerance may develop mainly in subjects with insulin re sponses below given threshold or if the probability to develop diabetes is inversely correlated to the magnitude of the insulin response The latter alternative would be similar to the one relating the diastolic blood pressure to the incidence of cardiovascular accident

The finding of low insulin response also in children favors the hypothesis that th phenomenon is either inherited or acquired early in life Although the similarity of the insulin responses within monozygotic twin pairs speaks in favor of the former suggestion it is still possible that non-genetic influences during early life determine the responsiveness of the islets. On the other hand recent studies on the heritability of the insulin response in a large group of healthy subject indicates that genetic factors play a dominating role in determining the responsivenes of the β-cells.

The diminished insulin response of the diabetic and prediabetic subjects is a relative one since a near-normal response can be induced by the administration of very large amounts of glucose The deficiency of the β-cell can best be explained in the terms of a decreased sensitivity to glucose as an initiator of insulin secretion. Studies with normal islets indicate that the signal for thi initiation may be medi ated by augmentation of the cellular content of cyclic AMP after interaction between glucose and putative cell membrane receptor In an islet deficient in insulin response (Acomys cahirinus) the effect of glucose on cyclic AMP accumulation was markedly decreased. Thus, it is possible that low insulin response in the diabetic yndrome is due to reduction of the ability of glucose to augment cyclic AMP generation in the β-cell.

Glucose apart from its acute insulin releasing effect has the capacity to potentiate the re sponsiveness of the slets in a time dependent manner Indirect evidence suggests that this potentiation generated by a process related to the utilization of the hexose in the β-cell In low insulin responders and patients with mild diabetes the potentiating ability of glucose was found to be normal Based on such results it is suggested that, in the diabetic syndrome only the signal for initiation of insulin release is impaired while the β-cell still retains its ability to recognize glucose (and other agents) as potentiator of this signal.

The presence of normal glucose tolerance in

Table 4
IVGTT and insulin concentration (M±SD) in healthy subjects and patients with myocardial infarction
(From Christiansen et al 1968)

Subjects	n	IVGTT	Fasting	Insulin release		
				10	30	60
Healthy subj.	27	k >1 1	19 6± 5 1	60.7±22.0	44 0±1..0	29.2± 9 9
Myocardial infarction	12	k <1 1	23 7± 5 5	42.2±12.6	41.8±10.8	38.8±10.8
	13	k >1 1	31.5±13.3	102±84	69.2±39 0	44.5±14.5

*p <0.05

The above data in the literature taken together give a rather confusing picture of the relationship between latent diabetes and arteriosclerotic vascular disease However the data of Christiansen et al (1968) and of Boden (1971) although obtained in small numbers of subjects indicate that latent diabetes is much more common in coronary heart disease than in the normal population Furthermore Elkeles et al (1971) were able to demonstrate that in diabetics not requiring insulin there were more vascular complications among those with a poor insulin response after oral glucose than among those with a *moderate insulin response*

These studies do not give a definite answer to the question whether the decrease in glucose tolerance is a primary event or secondary to the vascular disease Therefore there is a great need for *retrospective* studies on the relationship between e g coronary heart disease and peripheral vascular calcifications on the one hand and prediabetes and latent diabetes on the other In addition *prospective* studies concerning the later appearance of the above vascular disorders in previously diagnosed prediabetics and latent diabetics are of paramount importance

Table 5
IVGTT early (0-20 min) and late (20-120 min) integrated serum insulin response to intravenous glucose in patients with myocardial infarction (Mean±SEM)
(From Boden 1971)

Subjects	IVGTT	n	ΣIRI μU/min/ml	
Healthy subj.	k >1 00	12	1548±159	3927±538
Myocardial infarction	k ≤1 00	7	744±116	2610±314
	k >1 00	10	1516±203	4040±490

*p <0.05 p <0 01

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non-diabetic low insulin responders suggests that the efficiency of endogenous insulin is augmented in such subjects It has been demonstrated that the sensitivity also for exogenous insulin is increased Studies with liver vein catheterization indicate that the liver is a major site of the augmented insulin sensitivity It was found that the basal glucose output was decreased in low insulin responders Furthermore minor elevation of the blood glucose level was sufficient to suppress completely glucose production In patients with overt diabetes hepatic glucose output was uninfluenced by glucose administration. It is proposed therefore that the hepatic hypersensitivity to insulin protects the low insulin responders from developing glucose intolerance The loss of such a compensatory mechanism may be one of the factors that precipitate diabetes in low insulin responders However the possibility remains that in low insulin responders further diminution of the secretory capacity of the islets with age decompensates the hepatic glucose balance in spite of unchanged sensitivity to insulin.

In the light of the above discussion on insulin sensitivity it becomes obvious that metabolic states accompanied by insulin resistance may easily disturb the control of glucose homeostasis if the β-cells are unable to drastically augment insulin secretion. The finding of a relatively low insulin response in obese subjects and acromegalic patients presenting glucose intolerance – in contrast to the striking hyperresponsiveness of such subjects with normal glucose tolerance – supports this statement

Although only a limited number of low insulin responders may be expected ever to develop diabetes this prediabetic state anyhow carries metabolic consequences (Cerasi and Luft, 1972) Thus the lipolytic response to exercise was shown to be of the diabetic type in a number of prediabetics. Furthermore metabolic derangements were as common in the infants of prediabetic mothers as in those of well controlled diabetics Finally the observation of a high frequency of glucose intolerance in patients with degenerative cardiovascular disease focuses the attention on a possible connection between low insulin response and arteriosclerosis Obviously this is a field awaiting exploration

The condition discussed in this review concerns a considerable part of the population, about 2 % have manifest diabetes at least 5 % latent diabetes and 15-20 % are low insulin responders Measures must be sought by which the deficiency of the β-cells common to all stages of the diabetic syndrome can be corrected Such measures may have an impact reaching far beyond the therapeutics of diabetes

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Application of radioimmunologic methods to problems in insulin antigenicity and hormonal assay

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Introduction

Twenty years ago it was questionable whether or not insulin was generally antigenic in man. In immunized guinea pigs, insulin sensitizing (Barral and Roux 1931 Lewis, 1937 Bernstein, et al. 1938 and Wasserman and Mirsky 1942) and complement-fixing (Wasserman et al 1940 Wasserman and Mirsky 1942) antibodies had been demonstrated as well as the production of insulin-neutralizing antibodies which were capable of inducing diabetes by passive transfer to mice (Moloney and Coval 1955) Nonetheless, evidence of formation of antibodies to insulin in man had only rarely been presented usually by the demonstration of insulin-neutralizing activity in serum (Banting et al 1938 Lerman 1944 Lowell 1942 Loveless 1946 Yankelowitch et al. 1956) or in serum globulin fractions (DeFilippis and Iannaccone 1952 Sehon et al 1955 Colwell and Weiger 1956) In fact, in 1955 or after more than thirty years of widespread use of insulin therapy for the clinical management of diabetes, Moloney and Coval had concluded (Moloney and Coval 1955) that "There is of course acquired resistance to insulin in diabetes which in some cases is due to antibodies t heterologous insulin. Fortunately this phenomenon relatively rare Smelo (1948) found that total of fifty-four cases of resistant diabetics had been reported in the English literature It would appear then, that insulin is poor antigen in diabetic humans." I 1956, Heidelberger wrote "Insulin is not ordinarily antigenic Ordinarily when injected into animals it does not give rise to antibodies which can be demonstrated by complement fixation or by the usual precipitation tests.

It was at this time that we (Berson et al., 1956 a, Berson et al 1956 b) introduced radioisotopic methods of high sensitivity for the detection of non-precipitating antigen-antibody complexes and thus were able to demonstrate that in all human subjects insulin-binding antibodies developed within a few weeks following the institution of insulin therapy Investigation of the insulin-antibody reaction in vitro revealed that the reaction was reversible and that in the presence of increasing concentrations of insulin the fraction of insulin bound to antibody decreased (Berson et al. 1956 b). It was on this observation that the radioimmunoassay of plasma insulin was based. However investigations which lasted for several years and which included studies on the quantitative aspects of the reaction between insulin and antibody (Berson and Yalow 1957 Berson and Yalow 1959 a) and the species specificity of the available antisera (Berson and Yalow 1959 b) were required to translate the theoretical concepts of radioimmunoassay first to the measurement of plasma insulin in rabbits following exogenous insulin administration (Berson and Yalow 1958) and then to the measurement of insulin in unextracted human plasma (Yalow and Berson, 1959· Yalow and Berson, 1960 a Yalow and Berson 1960 b).

Introduction of radioisotope techniques not only produced an orders-of-magnitude improvement in analysis of immunologic problems but resulted in an equally dramatic improvement in the assay of hormones in plasma. Attempts had been had earlier to detect circulating plasma insulin using in vivo bioassay i.e. by measurement of the reduction of blood sugar following the injection of plasma into animals sensitized to the action of small amounts of insulin. However the sensitivity of the method was so low and the techniques proved to be so onerous that in the 1950's investigators employed instead some type of in vitro bioassay i.e. the measurement of the effect of plasma on some aspect of glucose metabolism in excised muscle or fat tissue (See reference – Yalow and Berson 1960 c for review). Although in some laboratories the fat pad and diaphragm bioassays provided

Insulin antigenicity

1 Detection of antibodies

Since it is now generally accepted that insulin-antibodies are found in the plasma of all subjects treated with commercial preparations of beef-pork insulin, it is of interest to consider why their universal presence was not recognized for more than 30 years. The answer lies in the observation that the insulin-antibody complex in human plasma rarely if ever spontaneously precipitates, does not fix complement, and is generally of such low titre that it is not detectable by hemagglutination or hemagglutination-inhibition methods. Nonetheless insulin-binding antibodies are readily detectable using radioisotopic methods that is by incubation of radioiodine-labeled insulin in plasma followed by appropriate separation of the radioiodinated insulin which antibody has bound (B) from that which is unbound and free (F). The first separation methods employed included paper electrophoresis and chromatoelectrophoresis (Berson et al 1956 b). In common with many other peptidal hormones insulin in its free state tends to absorb firmly to paper. In contrast it does not do so when bound to antibody. Therefore if an incubated mixture of labeled insulin and plasma is applied to a strip of filter paper for electrophoresis or chromatoelectrophoresis, the labeled insulin in the plasmas of untreated subject remains at the site of application i.e. the origin, whereas in the plasma of treated patients in whom antibody has developed, the insulin bound to the antibody moves between the gamma and the beta globulins (Fig 1). On chromatoelectrophoresis, the serum proteins do not separate significantly from each other but within a matter of only 20 to 30 minutes, all of the serum proteins carrying the insulin-antibody complexes have migrated away from the origin. (Fig. 1). On starch block electrophoresis labeled insulin in the immune plasma remains with the inter-beta-gamma globulins close to site of application and the free insulin which is not absorbed to the starch has an electrophoretic mobility almost that of albumin (Fig. 1)

A wide variety of other separation methods have since been used. These include: adsorption of free insulin to other solid phase materials such as uncoated (Palmieri et al. 1971) or coated charcoal (Herbert et al 1965) talcum powder or other silicates (Rosselin et al. 1966) precipitation of antigen-antibody complexes by antibodies to human gamma-globulin (Skom and Talmage 1958) salting out techniques (Gordis, 1960) including use of organic solvents adsorption or complexing of antibody to solid phase material such as glass, styrene (Catt et al. 1966) or Sephadex (Wide and Porath, 1966) or the use of sieving systems that retard free insulin relative to the insulin-antibody complexes (Genuth et al. 1965)

The binding of insulin to antibody in vivo leads to the retention of insulin within the blood stream and extracellular fluids (Berson et al 1956 b). This results in the protection of insulin while bound from the insulin degrading mechanisms of the body and therefore tends to prolong the action of insulin. It also blocks the effect of insulin on tissue cells, so that for a given administered dose the blood sugar-lowering effect of the insulin is reduced. *As the insulin-antibody complex* dissociates, some of the insulin gains access to the cells and some recombines with antibody. To analyze completely the role of insulin antibodies in modulating the action of insulin requires knowledge of the total binding capacity of the antiserum the distribution of binding sites between those with low and those with high energy and the rates of association and dissociation of insulin-antibody complexes

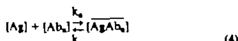
reasonable results concerning the levels of circulating plasma insulin in the fasting state *and after glucose feeding these methods* have not been employed in recent years in studies on the regulation of insulin secretion The first assay of insulin using immunologic methods employed the technique of he magglutination inhibition i e the inhibition of hemolysis of sensitized erythrocytes It was not capable of detecting less than about 0 1μg (2500 U) of beef insulin and therefore was completely useless for measuring plasma insulin in man or animals (Arquilla and Stavitsky 1956) Soon after its development (Berson and Yalow 1957 Berson 1957 Berson and Yalow 1958 Yalow and Berson 1959 Yalow and Berson 1960 a Yalow and Berson 1960 b) radioimmunoassay became the assay method of choice and most of our *present knowledge of the mechanisms invol*ved in the regulation of insulin secretion in normal and pathologic states has been obtained since 1960 with the use of radioimmunoassay methodology

This chapter attempts to survey some aspects of the application of radioimmunologic methods to problems of the antigenicity of insulin and to the measurement of insulin and related peptide hormones in man

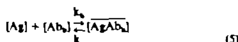
$$\frac{B}{F}=K([Ab^{\circ}]-B) \qquad (3)$$

The ratio of bound to free insulin is a linear function of the concentration of bound insulin and a plot of B/F vs B yields a straight line with a slope of K, (where K is the equilibrium constant of the reaction) and an ordinate intercept of K[Ab°] (Fig. 2 left). The concentration of antibody binding sites is determined from the extrapolation of the line to its intercept on the horizontal axis, since [Ab°] = B at B/F = O

Generally a plot of B/F vs B is curvilinear (Fig. 2 right) and this can be attributed to the reaction of univalent insulin with two or more orders of antibody binding sites (Ab_a and Ab_b) each *with characteristic equilibrium* constants, then



$$[Ag]+[Ab_a]\underset{k}{\overset{k_a}{\rightleftarrows}}[\overline{AgAb_a}] \qquad (4)$$



$$[Ag]+[Ab_b]\underset{k}{\overset{k_b}{\rightleftarrows}}[\overline{AgAb_b}] \qquad (5)$$

and by rearrangements similar to those of the preceding equations it follows that

$$\frac{B}{F}=K_a([Ab^{\circ}_a]-B_a)+K_b([Ab^{\circ}_b]-B_b) \qquad (6)$$

where $B = B_a + B_b = [\overline{AgAb_a}] + [\overline{AgAb_b}]$ and K_a and K_b are the respective equilibrium constants. The shape of the B/F vs B curve is dependent on the relative values of the K and [Ab°]'s. In the experimental situation shown in Fig. 2 (right), the antibody concentration and equilibrium constants for the two sites were obtained by curve fitting. The total concentration of antibody binding sites ($Ab^{\circ}_a + Ab^{\circ}_b$) is again determined from the intercept on the horizontal axis.

Transient-state kinetic studies (Berson and Yalow 1959) have also been used to demonstrate that insulin combines with at least two antibody binding sites with very different rates of reaction (Fig. 3). On mixing of insulin in antiserum there is a complex formed very rapidly but this complex also dissociates rapidly i.e., at a rate of 3 to 25 per cent per minute. After some time minutes or hours depending on the particular antiserum, most of the insulin is found in a complex that dissociates quite slowly i.e., at a rate of 1 to 60 per cent per hour. The relative proportion of rapidly and slowly dissociating complexes that exists at equilibrium is characteristic of a particular antiserum and is unchanged by dilution of that antiserum. This observation is consistent with a model of monovalent insulin reacting with two orders of antibody sites and not with a model of divalent insulin for*ming large molecular weight* complexes (Berson and Yalow 1959 a).

The observed kinetic constants for the reaction of beef insulin with specific antibody in different antisera are given in Table I. The minimal concentration of antibody binding sites detectable by these methods is of the order of 10^{-8}M

The theoretical model just described for reaction according to the law of mass action of univalent insulin with two or more orders of antibody combining sites predicts that the observed B/F ratio should be a monotonically decreasing function of antigen concentration. Dose-response curves consistent only with this prediction have been reported for the reaction of insulin with human anti-insulin sera (Fig. 2). However Matsukura et al. (1971), Matsuyama et al (1971) and Weintraub et al. (1973) have reported that in studies employing animal antisera to ACTH or to HCG the dose-response curves have been anomalous i.e. the B/F ratio first increases with low increments in hormonal concentration and then decreases. The appearance of such curves has given rise to the descriptive term, "hook effect" This effect has been observed with several guinea pig antisera obtained early in the course of immunization

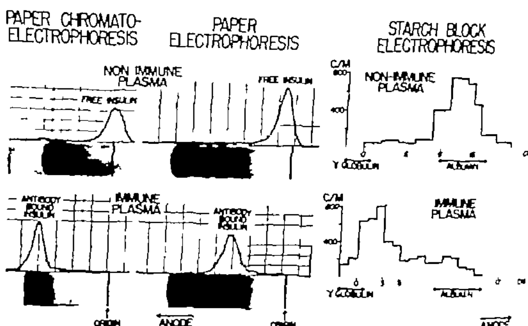


Fig 1
I^{131}-Insulin was added to the plasmas of insulin-treated (bottom) and untreated (top) human subjects and the mixtures were applied to a starch block (right) or to paper strips (middle) for electrophoresis or to paper strips for hydrodynamic flow chromatography combined with electrophoresis (left) After completion of electrophoresis segments were cut out of the starch block for assay of radioactivity and the paper strips were assayed in an automatic strip counter The zones of migration of albumin and γ-globulin were identified on the starch block by running samples containing I^{131}-albumin and I^{131} γ-globulin on the same block (Reproduced from Berson and Yalow In The Harvey Lectures Series 62 Academic Press 1968 p 107

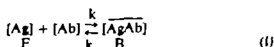
2 Quantitative aspects of the insulin-antibody reaction

Quantitative evaluation of the concentration of antibody-binding sites and the kinetics of the antigen-antibody interaction is facilitated by monitoring the reaction with radioiodine labeled insulin (Berson and Yalow 1959 a) It is required that labeled and unlabeled insulin behave identically in the immune system for this approach to be valid (See Appendix A for method of preparing radioiodine-labeled insulin with suitable characteristics)

Consider the law of mass action to be applicable. The reaction between univalent insulin (Ag) and a single order of antibody binding sites (Ab) may then be formulated as follows

$$\underset{F}{[Ag]} + [Ab] \underset{k}{\overset{k}{\rightleftarrows}} \underset{B}{[\overline{AgAb}]} \qquad (1)$$

Here F = [Ag] the molar concentration of the free uncomplexed antigen B = $[\overline{AgAb}]$ the molar concentration of complexed antigen or of antibody combining sites and [Ab] is the molar concentration of uncomplexed antibody combining sites Then if K is the equilibrium constant and $[Ab^o]$ is the molar concentration of total antibody combining sites so that $[Ab^o] = [Ab] + [\overline{AgAb}]$ we have from Eq (1)



$$K = \frac{k}{k} = \frac{[\overline{AgAb}]}{[Ag]\,[Ab]} = \frac{B}{F([Ab^o] - [\overline{AgAb}])} = \frac{B}{F([Ab^o] - B)} \qquad (\;)$$

Thus

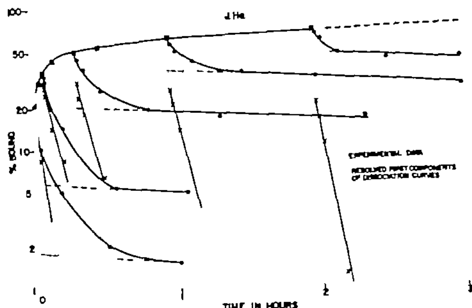


Fig 3
Experimental determination of th rat of association and dissociation of insulin-antibody complexes The curv joining the solid squares epresents the rate of binding of tracer I^{131}-in ulin At 0.5 5 20 60 and 120 minutes aliquots were emoved to tubes containing swamp g amounts of nlabeled insuli The curv s joining th solid circles represent the rate of dis ociation of the I^{131}-insuli bound t these times The latter curves were resolved into two component by extrapolati g the terminal segment to the times marking onset of dis ociation (R produced from Berson and Yalow 1959 a)

the titer of sera obtained after one or two immunizing doses is too low to make such studies feasible

3 Species specificity

Of interest with respect t the variable affinity of insulin for antibody is the species specificity of insulin in it reaction with antibody (Berson and Yalow 1959 b) Some human beef-pork antisera distinguish poorly among different mammalian insulins and appear to react similarly with most of them (Fig. 6 left) Other antisera appear more sensitive to small differences in amino acid sequences. Where distinction is made the order of reactivity of insulins from the four ungulate species is generally beef > sheep > pork > horse (Fig. 6 right) Since beef and sheep insulins differ only in the 9th amino acid of the A chain (A9) and pork and horse insulins also differ from each other only in this position but differ from beef and sheep insulins also at A8 and A10 (Brown et al 1955 Harris et al. 1956) we concluded that beef insulin was probably the principal antigen in the mixture (Berson and Yalow 1959 b) This would explain the stronger reactivity of sheep insulin (which is not present in commercial insulins) than of pork insulin The different reactivities of the various insulins which differ only in one or more of the amino acids in the 8-10 region of the A chain suggested that this region is probably involved in the binding to antibody

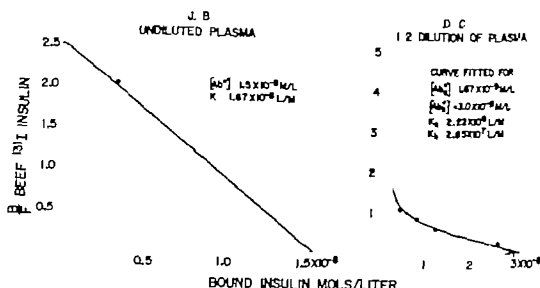


Fig 2
B/F vs B curves for nonresistant insulintreated subjects
Left – Curve fitted for univalent insulin reacting with a single order of antibody combining sites
Right – Curve fitted for univalent insulin reacting with two orders of antibody combining sites (Data reproduced from Berson and Yalow 1959 a)

when the antisera are used at low dilution but not at high dilution (Fig 4) (Cresto and Yalow 1974) The hook effect does not arise from possible artifacts introduced by various methods used to separate free from antibody-bound hormone or from differences between labeled and unlabeled antigen and has never been observed with the Fab fragments obtained following papain digestion of the immune gamma globulin component Although others (Weintraub et al 1973) have suggested that the hook effect arises because the complexing of one antigen molecule might enhance the energy of binding of another antigen molecule to a second antibody site on divalent antibody (cooperativity) we have concluded from a study of the kinetics of reaction of insulin with a guinea pig antiserum that in this situation at least another explanation is more likely When the anti-insulin serum was used at a dilution of 1 10000 equilibration of the reaction resulting in an anomalous standard curve required at least 4 days (Fig. 5) When the same antiserum was used at a dilution ten-fold greater the usual monotonically decreasing standard curve was observed and equilibration was achieved even earlier (Figs 4 & 5). This behavior i e a longer period required for equilibration in spite of higher concentrations of antibody is more consistent with a slow association constant and a virtually irreversible reaction than with the enhanced association rate predicted by cooperativity Furthermore our observations and those of Imura et al (1974) that the hook effect usually can be demonstrated only with antisera obtained early in immunization schedule and is less prominent or disappears after subsequent immunizations seem to suggest that 19 S globulins may be implicated We therefore hypothesize that the hook effect may arise from the slow aggregation of larger but still non-precipitating complexes of antigen with 19 S globulins rather than to cooperativity That failure to detect a hook effect with human antisera to insulin may be due simply to the low antigenicity of insulin in man so that

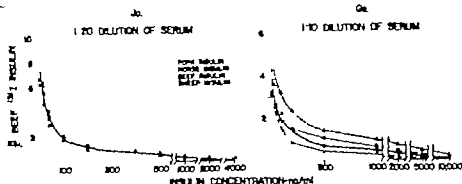


Fig. 6
Ratio of bound to free (B/F) beef ¹³¹I-insulin as a function of concentration of various unlabeled insulins in discriminating (right) and non-discriminating (left) antisera (Data reproduced from Berson and Yalow 1959 b)

pork insulin is more antigenic than regular crystalline pork insulin suggests that administration in the longer acting form enhances the antigenicity at least, even if it is not the direct cause.

The observation that porcine insulin preparations are generally less antigenic in man than those containing bovine insulin has been confirmed by a number of independent investigators (Devlin and Dugan, 1969; Schlichtkrull et al. 1972; Andreani et al. 1972; Andersen 1973 a; Deckert et al. 1974).

A seemingly inexplicable early finding was the ability of certain human antisera to detect immunologic differences between insulins purported to have the same amino acid sequences. Thus we showed that a few human antisera recognized pork and whale insulins as immunologically distinct (Berson and Yalow 1961) although their amino acid sequences were reported to be identical (Harris et al. 1956; Ishihara et al. 1958). We concluded that the distinguishable aspects of the two insulins might reside in certain conformational features related to unknown differences in their secondary and tertiary structures. However, since structural alterations or other artifacts may have occurred during extraction and purification, similar studies with native unextracted insulins appeared to be necessary. When Smith (1961, 1966) showed that dog insulin had the same amino acid sequence as pork and sperm whale insulins, it was practical to intercompare the relative immunoreactivity of dog and pig *plasma* insulins. The concentrations of insulin in the animal plasmas were determined with a guinea pig antiserum that does not distinguish be-

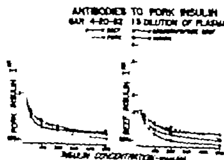


Fig. 7
Ratio of bound to free (B/F) pork ¹³¹I-insulin (left) and beef ¹³¹I-insulin (right) as a function of various unlabeled insulins in a human antiserum developed after 11 months of therapy with pure porcine NPH insulin. (Reproduced from Berson and Yalow 1963)

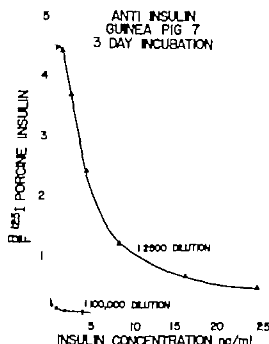


Fig 4
Standard curves for radioimmunoassay of insulin The anomalous curve is obtained in marked antibody excess (1.2500 dilution of antiserum) but not when the same antiserum is used at high dilution (1 10000) (Reproduced from Cresto and Yalow 1975)

and a stronger reaction of labeled beef than of labeled pork insulin with the new antibodies generated (Berson and Yalow 1963) Furthermore we observed that prolonged administration of regular crystalline pork insulin only occasionally results in detectable antibodies in human subjects (Berson and Yalow 1970 a) Since pork (Brown et al., 1955) and human (Nichol and Smith, 1960) insulins differ only in the amino acid at the carboxyl end of the B chain we considered that the site of antigenicity of pork insulin in man might reside in this region However the observation that desalanine pork insulin and desoctapeptide beef insulin react well with human antibodies to NPH pork insulin (Fig. 7) (Berson and Yalow 1963) suggest that the antigenicity probably resides other than in the C terminus of the B chain. That NPH and probably represents a site of antigenicity of commercial insulin in man

Therefore one would predict that commercial pork insulin might be less antigenic than commercial beef or beef-pork mixtures. This was confirmed by our finding that although NPH porcine insulin was antigenic in all subjects treated the antibody concentration was generally quite low (Berson and Yalow 1963) The antibodies so generated reacted more strongly with labeled pork than with labeled beef insulin (Fig. 7) although in some antisera there was no significant difference in the competitive inhibition of several unlabeled insulin species for the binding of labeled pork insulin to antibody In one subject treated with NPH pork insulin for almost a year treatment with NPH beef insulin resulted in a marked increase in antibody concentration

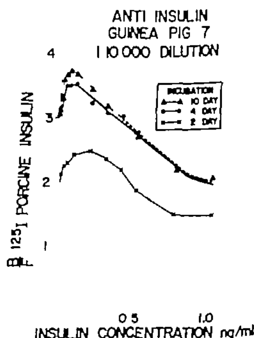


Fig 5
Standard curves for radioimmunoassay of insulin Separation of antibody-bound from free labeled hormone was effected by charcoal after the indicated periods of incubation at 4°C (Reproduced from Cresto and Yalow 1975)

Dr J Schlichtkrull commercial regular U-40 pork insulin (Lilly) and Lilly Research crystalline pork insulin Lot No 88194 all behaved identically However Lilly Research crystalline pork insulin Lot No 499667 reacted more strongly than did the other preparations in certain antisera. It seemed to us that the explanation could be that No. 499667 might have been contaminated with some beef insulin. Dr Otto Behrens of the Eli Lilly Research Laboratories confirmed that at the time this lot was processed the preparation of beef and pork insulins were not completely separated and that such accidental contamination might have occurred. In addition one must consider the possibility that even if at the pharmaceutical laboratory completely separate facilities were used for extraction and purification of beef and pork insulins this would not exclude some laxity in monitoring the separate collection of pancreases from the two species. Certainly if beef insulin were to react more strongly in antisera generated in response to administration of presumably pure pork insulin, the probability of accidental contamination of the pork insulin with beef would be strongly suggested. If pork insulin reacts significantly more strongly than beef insulin, then it most likely that pork insulin was the immunizing antigen. When the immunoreactivity of beef and pork insulins are similar one could not be certain of the nature of the immunizing antigen. It would have been of interest in studies recently reported (Root et al 1977) on the relative antigenicity of USP porcine and single component porcine insulin to have studied the kinetics of the insulin-antibody reaction with labeled beef as well as with labeled pork insulin. Although the increased antigenicity of the USP porcine insulin compared to monocomponent porcine insulin was attributed to the heterogeneous forms in the former the possibility of contamination with beef insulin was not considered and therefore cannot be excluded.

The experience of the vast majority of investigators over the past 50 years is consistent with the view that insulin-binding antibodies develop only in response to treatment with exogenous insulin. Nonetheless there have been occasional reports which demonstrate the existence of insulin-binding antibodies in patients who present with hypoglycemia, who are presumed to have an insulinoma, and who deny a previous history of insulin therapy (Folling and Norman 1972 Ohneda et al. 1974). Our own experience in this area is limited to two cases. One is that of a nurse (patient of Dr Melvin Horwith Cornell Medical School N Y) who presented with hypoglycemia. Repeated plasma insulin assays were performed in the course of several months hospitalization and we noted the gradual appearance of insulin-binding antibodies. Surreptitious self-administration of insulin was suspected and was subsequently confirmed by the finding of an insulin syringe concealed within a sanitary napkin in her bedside table The second case had a 10 year history of repeated hypoglycemic episodes necessitating multiple hospitalizations Several years before his admission to our hospital surgical intervention appeared indicated an adenoma was not found and a partial pancreatomy was performed at another institution. His diabetes was usually well-controlled with small doses of insulin but on occasion he had severe hypoglycemic episodes generally after an overnight fast when insulin and breakfast was withheld prior to glucose tolerance testing. During one such episode we demonstrated that the large increase in circulating insulin was due to beef-pork not human insulin. These studies were performed with an antiserum that distinguishes human insulin from the animal insulins (Berson and Yalow 1959 b). The observations of Folling and Norman (1972) from Norway that pig insulin reacts more strongly than human or beef insulin with the antibodies from their patient and of Ohneda et al (1974) from Japan that beef insulin reacts more strongly than human or pork insulin with antibodies from their patient are not inconsistent with the species of insulins available for commercial use in their respective countries — pork insulin being

tween crystalline dog and pork insulins Our studies (Berson and Yalow 1966 a) revealed that dog plasma and dog crystalline insulins are immunologically identical with each other but are distinguishable in certain human antisera from pig plasma and crystalline pork insulins (Fig. 8) If the data on amino acid sequences were valid then dog and pork insulins must have conformational differences despite their identical amino acid sequences How this could have occurred is perhaps explicable by the subsequent discovery of proinsulin (Steiner and Oyer 1967) if one can assume that the configuration of the insulin molecule is determined at the time of its synthesis via its proinsulin precursor The amino acid sequences of pork and dog proinsulins are strikingly different (Chance et al 1968 Peterson et al 1972) and hence conformational differences between the two prohormones are not surprising. If the subsequent removal of the connecting peptide leaves the secondary and tertiary structures unaltered then pork and dog insulins in

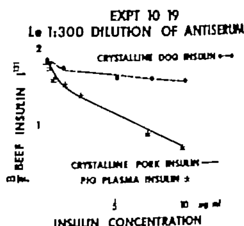


Fig 8 b
Experimental conditions are same as in figure 8 a Endogenous insulin concentrations in pig plasma were determined in guinea pig antiserum with crystalline pork insulin as standard (Reproduced from Berson and Yalow 1966 a)

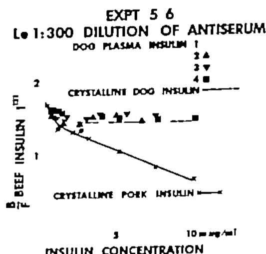


Fig 8 a
Cross reactivities of crystalline pork insulin and dog insulin and dog plasma insulin versus beef [131]I insulin a in a human antiserum Endogenous insulin concentratins in the dog plasma were determined by radioimmunoassay in guinea pig antipork insulin serum with crystalline dog insulin as standard

spite of identity of primary structure would remain distinguishable Studies on cleaving and recombining the A and B chains of dog and pork insulins are required to determine the preferred configuration in the absence of the directing effect of the C-peptide. If following recombination the pork and dog insulins maintain their different configurations then consideration must be given to the possibility that there may be errors, perhaps inversions in the reported amino acid sequences as for instance has recently been shown to be the case for human ACTH (Riniker et al 1972)

The immunologic specificity of some antisera permits great sensitivity in detecting differences in insulins not easily determinable in other systems Identity of reaction even with discriminating antisera has not been observed with a variety of beef insulins obtained from several sources (Berson and Yalow 1959 b). However some antisera appeared to distinguish among different pork insulins (Berson and Yalow 1966 a) Thus a pork insulin purified by Dr I Arthur Mirsky one supplied by Novo Laboratories through the courtesy of

Dr J Schlichtkroll commercial regular U-40 pork insulin (Lilly) and Lilly Research crystalline pork insulin Lot No 88194 all behaved identically However Lilly Research crystalline pork insulin Lot No 499667 reacted more strongly than did the other preparations in certain antisera. It seemed to us that the explanation could be that No. 499667 might have been contaminated with some beef insulin. Dr Otto Behrens of the Eli Lilly Research Laboratories confirmed that at the time this lot was processed the preparation of beef and pork insulins were not completely separated and that such accidental contamination might have occurred I addition one must consider the possibility that even if at the pharmaceutical laboratory completely separate facilities were used for xtraction and purification of beef and pork insulins this would not exclude some faulty in monitoring the separate collection of pancreases from the two species. Certainly if beef insulin were to react more strongly in antisera generated in response to administration of presumably pure pork insulin the probability of accidental contamination of the pork insulin with beef would be strongly suggested. If pork insulin reacts significantly more strongly than beef insulin then it is most likely that pork insulin was the immunizing antigen. When the immunoreact vity of beef and pork insulins are similar one could not be certain of the nature of the immunizing antigen. It would have been of interest m tudies recently reported (Root et al 1972) on the relative antigenicity of USP porcine and single component porcine insulin to have studied the kinetics of the insulin-antibody reaction with labeled beef as well as with labeled pork insulin. Although the increased antigenicity of the USP porcine insulin compared t monocomponent porcine insulin was attributed to the heterogeneous forms in the former the possibility of contamination with beef insulin was not considered and therefore cannot be xcluded

The experience of the vast majority of inves tigators over the past 20 years is consistent with the view that insulin-binding antibodies develop only in response to treatment with exogenous insulin. Nonetheless there have been occasional reports which demonstrate the existence of insulin-binding antibodies in patients who present with hypoglycemia, who are presumed to have an insulinoma, and who deny a previous history of insulin therapy (Folling and Norman 1972 Ohneda et al., 1974) Our own experience n this area is limited to two cases One is that of a nurse (patient of Dr Melvin Horwith Cornell Medical School N Y) who presented with hypoglycemia. Repeated plasma insulin assays were performed in the course of several months hospitalization and we noted the gradual appearance of insulin-binding antibodies. Surreptitious self-administration of insulin was suspected and was subsequently confirmed by the finding of an insulin syringe concealed within a sanitary napkin in her bedside table The second case had a 10 year history of repeated hypoglycemic episodes necessitating multiple hospitalizations. Several years before his admission to our hospital surgical intervention appeared indicated, an adenoma was not found and a partial pancreatomy was performed at another institution H s diabetes wa usually well-controlled with small doses of insulin but on occasion he had severe hypoglycemic episodes generally after an overnight fast when insulin and breakfast was withheld prior to glucose tolerance testing. During one such episode, we demonstrated that the large increase in circulating insulin was due to beef-pork not human insulin. These tudies were performed with an antiserum that distinguishes human insulin from the animal insulins (Berson and Yalow 1959 b) The observations of Folling and Norman (1972) from Norway that pig insulin reacts more trongly than human or beef insulin with the antibodies from their patient and of Ohneda et al. (1974) from Japan that beef insulin reacts more strongly than human or pork insulin with antibodies from thei patient are not inconsistent with the species of insulins available for commercial use in their respective countries – pork insulin being

more commonly used in Scandinavia than elsewhere From the apparent species specificity of the antibodies in these patients one can conclude that animal insulin is more likely to have been the immunizing antigen than is human insulin Identification with specific antisera of the species of insulin circulating during the time of hypoglycemic episodes is certainly important to evaluate whether surreptitious insulin administration is involved However at present, we conclude that the overwhelming evidence is against the development of autoantibodies to endogenous insulin.

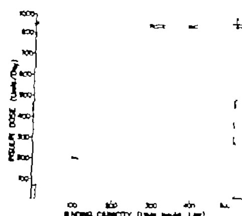


Fig 9
Insulin dosage and insulin antibody concentration expressed as insulin binding capacity in insulin-treated adult diabetic patients Shaded area 36 clinically nonresistant diabetics requiring less than 70 units insulin/day and insulin binding capacities less than 10 units/liter plasma 0 diabetics requiring 70 to 90 units insulin/day with insulin binding capacities varying from 22 to 35 units/liter plasma x clinically resistant patients (Reproduced from Berson and Yalow 1970 a)

4 Clinical aspects of insulin antigenicity

Although virtually every patient treated with commercial mixtures of beef pork insulin develops insulin-binding antibodies within a few months the total insulin binding capacity of such human antisera is usually quite low rarely exceeding 10-20 units insulin per liter and generally less than 1 2 units per liter (Berson et al 1956 b Berson and Yalow 1957 1959 a Deckert 1964 Berson and Yalow 1970 a) However most patients with chronic insulin requirements in excess of 100 to 150 units insulin per day have significantly higher binding capacities (Berson et al 1956 b Burrows et al 1957 Berson and Yalow 1959 a Yalow and Berson 1961 Berson and Yalow 1970 a) ranging from 60 to as high as 5000 units per liter (Fig 9). In all cases insulin binding capacities were determined as discussed earlier from extrapolation of the terminal portion of the curve of B/F vs B to the intercept on the horizontal axis i e when B/F = 0 From Fig. 9 it would appear that there is no strict correlation between antibody concentration and insulin dosage although patients with the highest binding capacities (>500 units/liter) generally have dosage requirements in excess of 300 units daily Other factors in addition to antibody concentration per se must be considered These include the kinetic factors involved in formation of the complex i e the equilibrium constant(s) for the reaction and the rates of association and dissociation of the complex compared to the rate of escape of insulin from extravascular space and the rate of elimination of insulin while still bound in the complex form and thus inhibited from exerting its physiologic effect Some estimates of the rate of immunologic wastage as a function of antibody concentration can be made (Table II). The underlying assumptions are that the space of distribution of the insulin-antibody complexes approximates that of gamma-globulin (~ 10 % of body weight – 7 liters in the average man) and the rate of plasma clearance of the complexes is about that of other soluble antigen-antibody complexes i e about 25 per cent per day (Weigle 1958). From these rough calculations it can be seen that in patients with high antibody concentrations insulin wastage can greatly exceed 100 units/day and in the patients with the highest antibody concentrations can exceed even 1000 units/day On the other hand in

the vast majority of insulin-treated subjects with antibody concentrations equivalent to less than 1-2 units insulin/liter Insulin wastage amounts to only a few units per day and would probably not be noticed In these non-resistant patients the effect of insulin antibodies would be most apparent in the intravenous insulin tolerance test where the immediate biologic effectiveness of the insulin would be lessened markedly because of its capture by antibody resulting in a picture of apparent insulin insensitivity

The energy of interaction of insulin with the different orders of antibody-binding sites in insulin-resistant patients is also an important factor is determining insulin dosage In the patient shown in Fig. 10 after 15 days of steroid therapy there was a striking decrease in the concentration of high-energy antibody with no significant decrease in the total binding capacity Associated with the decrease in the high energy antibody there was drop in daily insulin requirement from 400 U to 100 U It might well be that, in general the insulin requirements of insulin resistant patients

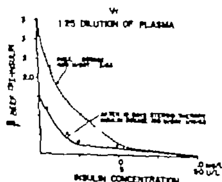


Fig 10

Ratio bound to free (B/F) beef [131]I-insulin as a function of insulin concentration in antisera obtained from insulin-resistant patient before and following 15 days of steroid therapy The therapy was accompanied by a reduction of daily insulin dosage from 400 U to 100 U

would be better correlated with concentration of high energy antibody binding sites rather than with total binding capacity The fact is that in resistant patients whose antibodies react significantly less well with pork than with beef insulin, a reduction in insulin requirements can generally be observed when pure pork rather than the beef-pork mixture is used for therapy (Berson and Yalow 1970 a Andreani et al. 1972)

Immune insulin resistance requiring dosages in excess of 200 U/day occurs rarely Although not statistically documented there is an impression that it has become more common during the past two decades The increased incidence if real, cannot be attributable to use of insulins of lesser purity but is probably due to alternation of therapy between insulin and oral hypoglycemic agents It is likely that continuous insulin therapy induces a state of immune paralysis with resultant low antibody concentrations. However a period without insulin followed by reinstitution of insulin therapy may then augment the antibody response thus leading, on occasion to insulin resistance. Since insulin resistance is uncommon there is probably no need for concern for the vast majority of insulin-treated subjects about the antigenicity of insulin. However were adequate supplies of low immunogenic pork insulin available for and used in the high risk group i e patients not under continuous insulin therapy the incidence of immune insulin resistance might be considerably reduced.

Since the lower immunogenicity of pork insulin is commonly accepted, serious consideration is merited as to whether it is economically feasible and medically desirable to have available for therapy only insulins from single species rather than mixed insulins For the foreseeable future the production of beef insulin will continue to exceed greatly that of pork insulin. It is then a question of more than rhetorical interest whether it is wise to dilute the available pork insulin into the beef

insulin pool and thereby reduce the supply of low immunogenic pork insulin

There has been a recent revival in studies on the *antigenicity of insulin* Root et al (1972) have suggested that certain higher molecular weight components in commercial insulin preparations may be largely responsible for this antibody formation and that the immunogenicity of *highly purified insulin* preparations is of a low order of magnitude This suggestion does not take into account the lesser antigenicity of pork than of beef insulin which as just discussed we had earlier described (Berson and Yalow 1963 Berson and Yalow 1970 a) and which recently has been reconfirmed in several studies (Andreani et al 1972 Schlichtkrull et al 1972 Deckert et al 1974)

It is generally accepted that peptides and proteins usually found in the circulation are not *antigenic in the host* Nonetheless autologous gamma-globulin which had simply been precipitated and frozen was shown to be antigenic in a rabbit (Milgrom and Witebsky 1960) purified human growth hormone has been shown to be antigenic in man (Roth et al 1964 Prader 1964 Touber and Maingay 1964) and a peptide as small as arginine vasopressin has been shown to be antigenic in the rabbit although it is the endogenous hormone in that species (Roth et al 1966) Thus through the years evidence has been accumulating that chemical purification of endogenous peptides can introduce some slight denaturation which renders them foreign and hence antigenic in the host

Root et al (1972) has found that proinsulin like components constituted about 6 % of a USP pork insulin sample studied Our own studies on small-scale extraction of individual human pancreases showed the proinsulin component to be less than 1 2 % (Yalow and Berson 1970 a) Steiner and Oyer (1967) observed that commercial preparations of crystalline insulin contained less than 1 per cent of material which behaved similarly to the proinsulin they detected in their biochemical studies Recently Andersen (1973 b) has found that recrystallized porcine insulin contained less than 2 % proinsulin It may well be that the higher concentration of proinsulin like components found in the USP preparation of Root et al (1972) was due to alterations in insulin which occurred during the processing. We have shown earlier that simply storing insulin including commercial insulin refrigerated or frozen results in alterations readily detectable on starch gel electrophoresis (Berson and Yalow 1966 b). To our knowledge there has been as yet no *investigation reporting on the characteristics* of the hormonal forms of insulin at the various stages in its extraction and purification for clinical use

The question can be raised as to whether proinsulin should be more antigenic than monocomponent insulin Proinsulin is usually found in the circulation (vide infra) and in fact generally constitutes a higher fraction of plasma insulin than of pancreatic insulin (Yalow and Berson 1970 a) Thus there is no a priori reason to expect porcine proinsulin to be more antigenic than porcine insulin Additional studies are required to determine whether it is proinsulin or insulin denatured by the chemical processing that is responsible for the reported increased antigenicity of the usual commercial insulin compared to monocomponent insulin (Root et al 1972 Schlichtkrull et al 1972 Deckert et al 1974)

Some reservations concerning the interpretation of the several reports suggesting the lesser immunogenicity of monocomponent insulin (Root et al 1972 Schlichtkrull et al 1972 Deckert et al 1974) have been introduced by the recent report of Tantillo et al (1974) who employed commercially available U 100 single-peak beef-pork insulin for their studies They found that diabetic patients previously untreated with insulin do form antibodies to this preparation and to the same degree as do patients

on the less highly purified standard USP insulin show no fall in antibody levels when treated with the single-peak beef-pork insulin. In fact two of the patients studied showed a rise in antibody levels after changing to single-peak insulin. They concluded that removal of insulin components of relatively high molecular weight is apparently not sufficient to reduce the antigenicity of beef-pork insulin in man.

The failure of Tantillo et al. (1974) to observe lesser antigenicity of commercial single-component beef-pork insulin preparations would appear to be discrepant with the finding of several groups (Root et al. 197? Schlichtkrull et al. 1972 Deckert et al. 1974) who have suggested that specially prepared single-component pork insulin is less antigenic than the usual USP pork insulin preparations of the same type. One cannot rule out the possibility that the single-peak insulin employed by Tantillo et al. (1974) still contained other components in the same molecular weight class as insulin, i.e. desamido insulins, etc. and that the antigenicity of the commercial preparation was due to these other components. Nonetheless an equally tenable hypothesis is that beef insulin itself is sufficiently antigenic in man so that the slight enhancement of its antigenicity by the presence of large molecular weight components is not detectable and that pork insulin is so weak an antigen that its immunogenicity must be enhanced by the presence of high molecular weight components or by incorporation into long acting therapeutic preparations for the effect to be manifest.

Still another aspect of the immunogenicity of insulin must be considered. When it was first appreciated that virtually all insulin-treated patients develop insulin-binding antibodies it was suggested that some of the late diabetic manifestations may be causally related to long continued exposure to circulating insulin-antibody complexes (Berson et al. 1956 b). Support for this concept arises from a number of studies including those by Wehner et al. (1973) who found basement membrane alterations in the capillaries of glomeruli from rabbits treated with (a+b) insulin components whereas no such changes were demonstrable in rabbits treated with monocomponent (M-C) insulin of approximately equal weight. Criticism of these studies can be directed in part to the observation that after four weeks treatment the prevalence of subepithelial basement membrane protuberances following injection of only the solvent for the (a+b) components was six times that following injection of the M-C insulin in its solvent so that one can on this basis alone question the specificity of the lesions. Furthermore at best (a+b) components represent only a small contaminant of commercial insulin and its significance in real-life situations can hardly be determined by administering it in marked excess in a solvent which in itself appears to be damaging. Gellman et al. (1959) suggested that their identification of γ-globulins in vascular lesions of diabetes supported the theory of the involvement of insulin-antibodies in the development of the lesion. The validity of this theory also appeared to be strengthened by the observations of Berns et al. (196.) that conjugated insulin was bound in vitro to the same sites in the vascular walls where γ-globulin localized in vivo and that conjugated anti-insulin serum was bound in vitro in similar sites as conjugated insulin. These reports were questioned on a methologic basis by Larsson (1967) whose investigations of skin biopsies studied with conjugated pig insulin seemed to indicate that the "binding" of insulin to dermal vessel walls is dependent on the fixation methods used in the preparation of the sections studied and who concluded that immunohistochemical methods lend no *support to the concept that* the vascular lesions of diabetics are derived from immunologic reactions with insulin.

On a clinical basis the most cogent criticisms of the theory of a causal relationship between the renal lesions of diabetics and exposure to insulin-antibody complexes comes from an

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The question can be raised as to whether proinsulin should be more antigenic than monocomponent insulin Proinsulin is usually found in the circulation (vide infra) and in fact generally constitutes a higher fraction of plasma insulin than of pancreatic insulin (Yalow and Berson 1970 a) Thus there is no a priori reason to expect porcine proinsulin to be more antigenic than porcine insulin. Additional studies are required to determine whether it is proinsulin or insulin denatured by the chemical processing that is responsible for the reported increased antigenicity of the usual commercial insulin compared to monocomponent insulin (Root et al 1972, Schlichtkrull et al 1972 Deckert et al., 1974)

Some reservations concerning the interpretation of the several reports suggesting the lesser immunogenicity of monocomponent insulin (Root et al 1972 Schlichtkrull et al 1972 Deckert et al 1974) have been introduced by the recent report of Tantillo et al (1974) who employed commercially available U 100 single-peak beef-pork insulin for their studies They found that diabetic patients previously untreated with insulin do form antibodies to this preparation and to the same degree as do patients

on the less highly purified standard USP insulin show no fall in antibody levels when treated with the single-peak beef-pork insulin. In fact t o of the patients studied showed a rise in antibody levels after changing to single-peak insulin. They concluded that removal of insulin components of relatively high molecular weight is apparently not sufficient to reduce the antigenicity of beef-pork insulin in man

The failure of Tantillo et al. (1974) to observe lesser antigenicity of commercial single-component beef-pork insulin preparations would appear to be discrepant with the finding of several groups (Root et al 1972, Schlichtkrull et al 1972, Deckert et al. 1974) who have suggested that specially prepared single-component pork insulin is less antigenic than the usual USP pork insulin preparations of the same type One cannot rule out the possibility that the single-peak insulin employed by Tantillo et al (1974) still contained other component in the same molecular weight clas as insulin i.e desamido insulins etc and that the antigenicity of the commercial preparation was due to these other components. Nonetheless an equally tenable hypothesis is that beef insulin itself is sufficiently antigenic in man so that the slight enhancement of it antigenicity by the presence of large molecular weight components is not detectable and that pork insulin is so eak an antigen that it immunogenicity must be enhanced by the presence of high molecular eight component or by incorporation into long-acting therapeutic preparations for the effect to be manifest

Still another spect of the immunogenicity of insulin must be considered When it was first appreciated that virtually all insulin-treated patient develop insulin-binding antibodies it was suggested that some of the late diabetic manifestations may be causally related to long continued xposure to circulating insulin-antibody complexes (Berson et al. 1956 b) Support for this concept arises from a number of studies including those by Wehner et al (1973) who found basement membrane alterations in the capillaries of glomeruli from rabbits treated with (a+b) insulin components whereas no such changes were demonstrable in rabbits treated with monocomponent (M-C) insulin of approximately equal weight. Criticism of these studies can be directed in part to the observation that after four weeks treatment the prevalence of subepithelial basement membrane protuberances following injection of only the solvent for the (a+b) components was six times that following injection of the M-C insulin in its solvent so that one can on this basis alone question the specificity of the lesions Furthermore at best (a+b) components represent only a small contaminant of commercial insulin nd its significance in real-life situations can hardly be determined by administering it in marked excess in a solvent which in itself appears to be damaging Gellman et al (1959) suggested that their identification of γ-globulins in vascular lesion of diabetes supported the theory of the involvement of insulin-antibodies in the development of the lesion The validity of this theory also appeared to be strengthened by the observations of Berns et al (196.) that conjugated insulin was bound in vitor to the same sites in the vascular wall where γ-globulin localized in vivo and that conjugated anti-insulin serum was bound in vitro in similar sites as conjugated insulin These eport were questioned on a methologic basis by Larsson (1967) whose investigations of skin biopsies tudied with conjugated pig insulin seemed to indicate that the "binding of insulin to dermal vessel walls is dependent on the fixation methods used in the preparation of the sections studied and who concluded that immunohistochemical method lend no support to the concept that the vascular lesions of diabetics are derived from immunologic reactions with insulin.

On a clinical basis the most cogent criticisms of the theory of a causal relationship between the enal lesions of diabetics and exposure to insulin-antibody complexes comes from an

insulin pool and thereby reduce the supply of low immunogenic pork insulin

There has been a recent revival *in studies on* the antigenicity of insulin Root et al (1972) have suggested that certain higher molecular weight components in commercial insulin preparations may be largely responsible for this antibody *formation and that the immunogenicity* of highly purified insulin preparations is of a low order of magnitude This suggestion does not take into account the lesser antigenicity of pork than of beef *insulin which as just discussed we had* earlier described (Berson and Yalow 1963 Berson and Yalow 1970 a) and which recently has been reconfirmed in several studies (Andreani et al 1972 Schlichtkrull *et al* 1972 Deckert *et al* 1974)

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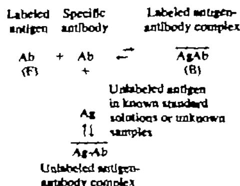
Radioimmunoassay

1 General considerations

The theoretical aspects of radioimmunoassay developed from the use of radioisotopic tracer techniques to study quantitatively the insulin-antibody reaction. The mathematical analysis required that the reactions of labeled and unlabeled insulin with the antibodies employed be identical (Berson and Yalow 1959 a). The principle employed in the studies on the quantitative aspects of the insulin-antibody reaction was simply that of isotope dilution. The classical isotope dilution principle first described by Hevesy in 1931 (Hevesy and Hobbie 1931) requires for the validation of the measurements 1) identical behavior of labeled and unlabeled substance 2) uniform mixing in the system to be analyzed, and 3) quantitative determination of labeled and unlabeled substances in a portion of the mixture to be analyzed. The dilution principle has been used extensively to measure the size of various body compartments such as blood volume, body water, exchangeable sodium and potassium, etc., as well as to determine the rates of transfer of labeled substances between these compartments. (See Yalow and Berson, 1956 for review)

In 1950 Haurowitz (1950) summarized the application of labeled antigen techniques which together with an analysis derived from the law of mass action permitted the study of the antigen-antibody *precipitin* reaction. The new dimension added by Berson et al. (1956 b) to these immunologic studies was the development of a methodology for measuring *soluble* complexes and which permitted quantitation of the primary reaction, i.e. the initial combination of antigen with antibody. The ability to measure the primary antigen-antibody reaction resulted in a conceptual advance in immunochemistry since it was not appreciated at that time that the association constant for any antigen-antibody reaction could be as high as $K = 10^{8}$ (Day 1966). The reason for such skepticism was partially contained in the Pauling theory of 1940, in which a much lower energy of interaction was predicted, i.e. an upper limit for K of 10^{5} (see Day 1966). In spite of the significance of this methodology to immunochemistry, it was per se simply an extension of isotope dilution, which differs in several fundamental ways from radioimmunoassay.

The radioimmunoassay principle can be formulated in the following fashion:



The unknown concentration in the test sample of the substance to be measured is directly obtained by comparing the inhibition of binding of labeled antigen to antibody in the presence of the unknown with the inhibition produced by a set of known standards (*Fig. 11*). In contrast to the requirements for isotope dilution, when using radioimmunoassay – *1) there is no* requirement that labeled and unlabeled substance be identical or behave identically in the system employed, 2) *there is no requirement* for uniform mixing of labeled and unlabeled material – in fact some assays (often called non-equilibrium assays) are designed specifically to *avoid uniform* mixing of labeled and unlabeled material, 3) the sole requirement is that both standards and unknowns behave identically in competi-

appreciation that the specific lesion nodular glomerulosclerosis was in fact found in patients who died in the pre-insulin era (Castleman 1959) and has been reported in patients previously unaware of their diabetes (Rifkin et al 1941 Freedman 1957) and who therefore were presumably never treated with insulin. The fact is that in the past 20 years the era of hypoglycemic agents the majority of diabetic patients have never been treated with insulin and these patients continue to manifest the complications of diabetes Therefore there is no substantial basis on which to indict the immunogenicity of insulin in this problem

At present there is no evidence that the low levels of insulin-binding antibody found in the vast majority of insulin-treated subjects are in fact deleterious Since antibody-bound insulin prolongs the action of insulin it might actually be protective by moderating the peaks and troughs of insulin action The incidence of insulin resistance is still quite low and might well be reduced even further if greater supplies of pork insulin uncontaminated with beef insulin, were made available for the group at high risk. Whether expensive modification of the processing of insulin to reduce its antigenicity would be of value to the vast majority of patients requiring insulin therapy is a moot question

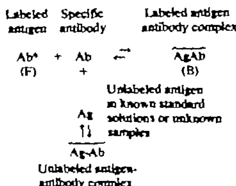
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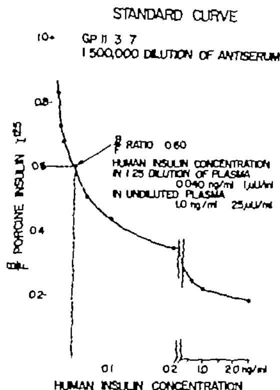


Fig 11
Standard curve for assay of human insulin Method of calculation of hormone concentration in unknown plasma is shown (Reprodu-

tively inhibiting the binding of the labeled substance

Radioimmunoassay (RIA) is similar to the isotope dilution methodology in that both employ labeled marker molecules yet the *fundamental assumptions and principles* are essentially different Some of the procedural aspects of RIA are considered in the Appendix It is important to appreciate that in RIA there is no requirement that standards and unknowns be biologically or chemically identical A necessary but not sufficient condition for the validation of a RIA procedure is that the antigen concentration in the unknown be independent of the dilution at which it is assayed This requires only *immunochemical* identity of standards and unknowns. This condition is tested by *making multiple* dilutions of an unknown sample and determining whether the competitive inhibition curve is superposable on the standard curve for assay or alternately whether the observed concentration in the diluted unknown plasma falls linearly with dilution (Fig. 12) When unknowns and standards are not chemically identical some antisera may distinguish between them and other antisera may not. *Some aspects of this problem were discussed earlier in the section on species specificity* Thus immunochemical identity or immunochemical potency *is dependent on the* characteristics of a particular antiserum In the application of RIA to the measurement of plasma and tissue insulin the use of the appropriate species insulin for standard generally assures immunochemical and biologic identity of standards and unknown However the discovery of proinsulin (Steiner and Oyer 1967) and the demonstration that many of the peptide hormones are heterogeneous in plasma and tissue (See Yalow 1974 for review) *might have made necessary identification* of the immunoreactive form(s) of insulin *when employing RIA for studies of the regulation* of insulin secretion in normal and pathologic states

2. *State of insulin in plasma and tissues*

Soon after the discovery of proinsulin (Steiner and Oyer 1967 Steiner et al 1969) *it was demonstrated by Roth et al* (1968) that in plasma there are immunoreactive components *of insulin* ("big insulin") with an elution volume on Sephadex gel filtration corresponding to that of proinsulin Since the immunologic potency of proinsulin as measured with many antisera is greater than its biologic potency as measured in several bioassay systems there was great concern that the measurement *of the concentration of* plasma insulin by radioimmunoassay would *not necessarily reflect the biopotency of the* circulating hormone In fact Roth et al (1968)



Fig 12
Effect of dil tion of plasma on measured concentration of endogenous plasma insulin Four replicate determinations were made for each point in the experiment on the right (Reproduced from Yalow and Berson 1960 b)

suggested that the physiology of insulin se cretion had to be reexamined completely especially in those conditions in man characterized by glucose intolerance with hyperinsulinism such as starvation, obesity and maturity-onset diabetes This suggestion was based on their finding that two hours after glucose administration, up to 50 % of the immunoreactivity in their assay corresponded to "big insulin (roth et al. 1968). Subsequent studies by this group (Gorden and Roth, 1969 Gorden et al, 1974) as well as by a number of other laboratories including our own (Yalow and Berson 1970 a Goldsmith et al. 1969 Rubenstein et al. 1968 Horwitz and Rubenstein, 1974) have confirmed that proinsulin-like material usually comprises only minor fraction of total immunoreactivity in the stimulated state in the typical radioimmunoassay system (Fig. 13) It should be noted that earlier studies on the fractionation of plasma by starch gel electrophoresis failed to reveal detectable differences between endogenous plasma insulin and crystalline insulin added to plasma (Berson and Yalow 1966 a). In this system insulin migrates in advance of serum albumin, while proinsulin which is larger and more basic than insulin is retarded migrating with about one-half the electrophoretic mobility of serum albumin. Thus had proinsulin been the major component in the plasmas studied the discovery of the heterogeneity of plasma insulin might have been made several years earlier (Berson and Yalow 1966 a)

In the fasting state in normal subjects and in various pathologic hypoinsulinemic states (Fig. 14) the fraction of the total immunoreactivity corresponding to proinsulin-like components is generally higher than in the period when stimulated insulin secretion occurs (Fig. 13) This is related at least in part to the reported slower turnover of proinsulin in man (half-time $T_{1/2}$ = 17.2 min) than of insulin (half-time $T_{1/2}$ = 4.8 min) (Starr et al. 1973) Consider for example, the space of distribution and the rate of transfer into that space to be the same for proinsulin and insulin and that after an extended non-secretory phase a single spurt of insulin containing proinsulin and insulin in a 1 4 ratio of respective molar concentrations is secreted. At the end of the spurt proinsulin would account for

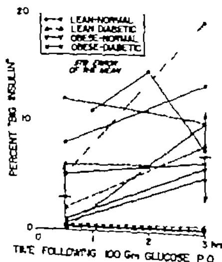


Fig 13
Per cent "big insulin as a function of time following oral glucose ingestion in lean or obese patients with normal or diabetic glucose tolerance t ts (Reproduced from Goldsmith et al 1969)

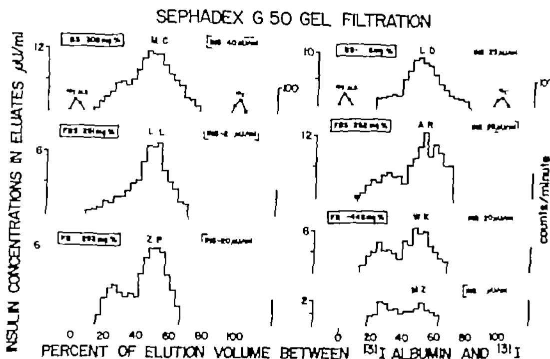


Fig 14
Sephadex G-50 gel filtration patterns of immunoreactive insulin in the plasma of diabetic patients with fasting hyperglycemia

20 % of the total immunoreactive insulin Since the rate of disappearance of the insulin is almost 4 times that of proinsulin then following the spurt the concentration immunoreactive insulin would decrease and the fraction of immunoreactivity circulating in the proinsulin form would increase so that for example at 20 min it would account for more than 2/3 the immunoreactivity Under steady state conditions at the same relative secretory rates the proinsulin component would account for about half the immunoreactivity

The observations that in some but not all cases of insulinoma an immunoreactive component having a Sephadex gel filtration volume corresponding to proinsulin may predominate in plasma (Goldsmith et al 1969 Lazarus et al 1969 Gorden et al 1971) can be explained at least in part by the fact that in some of these patients insulin concentrations in the non stimulated steady state might be high enough for fractionation The proinsulin insulin ratios in these plasmas would therefore be expected to be somewhat greater than those which are observed in non-insulinoma patients whose insulin concentrations in plasma are generally high enough to fractionate only following stimulation Nonetheless proinsulin-like components are a higher fraction of immunoreactive insulin in some insulinomas than in the normal pancreas (Yalow and Berson 1970 a) and therefore probably constitute a larger fraction of the immunoreactive insulin secreted from such insulinomas. The question as to whether the proinsulin-like component is in fact proinsulin of low biologic activity is relevant since insulinoma patients usually present with hypoglycemia and the insulin levels are not always markedly elevated Recently Gorden et al (1974) have demonstrated that the "big insulin component from the plasma of a non-tumor patient coincides with a ¹²⁵I-proinsulin marker and that the big insulin component from a patient with an islet cell carcinoma

was slightly but distinguishably less retarded. Furthermore they (Gorden et al. 1974) also find that the partially purified plasma proinsulin-like component from tumor subjects is less reactive than the proinsulin-like components from non-tumor subjects in radioimmunoassay and more reactive than porcine proinsulin when examined by radioreceptor assay. They interpret their data as suggesting that plasma proinsulin is not homogeneous and that the distribution between different components may be variable. Additional work certainly needs to be done to resolve this problem.

Proinsulin-like components are not the only larger immunoreactive component to appear in the plasma. We have demonstrated that in an unusual case of a patient with a suspected insulinoma and only occasional hypoglycemia in spite of inordinately high plasma immunoreactive insulin concentrations (600 micro units/ml fasting, 2,000 micro units/ml after glucose feeding) a new form of insulin, "big big" insulin, predominates (Fig. 15) (Berson and Yalow 1971, Yalow and Berson 1973 a). This component is thus virtually devoid of biological activity in vivo. It has an elution volume on Sephadex gel filtration smaller than that of labeled albumin and almost coincident with that of labeled γ-globulin (Fig. 16). This hormonal form of insulin maintains its integrity on refractionation, is immunochemically indistinguishable from the 6,000 mol. wt. insulin and is quantitatively convertible by controlled tryptic digestion to an insulin-like component (Yalow and Berson 1973 a). Big big insulin is found as a distinct component in extracts of insulinomas and normal pancreases, although it relative concentration is quite low, being less than 1 % of total immunoreactivity (Yalow and Berson 1973 a) (Fig. 15, Fig. 16). Whether or not this much larger hormonal form is the ultimate precursor of the insulin family has not been determined.

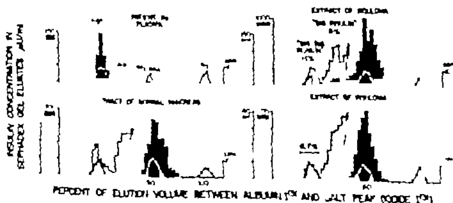


Fig. 15

Immunoreactive plasma insulin concentration (solid block columns) in Sephadex G-50 eluates of plasma of patient K. (top left), of an extract of a piece of normal pancreas (bottom left), and of extracts of two insulinoma (right). The concentrations in early eluates are also shown in simple on an expanded scale. (Reproduced from Yalow and Berson 1971)

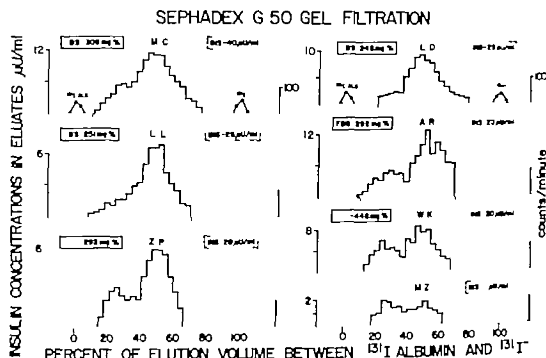


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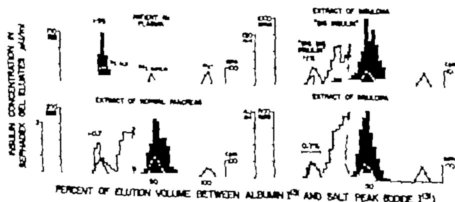


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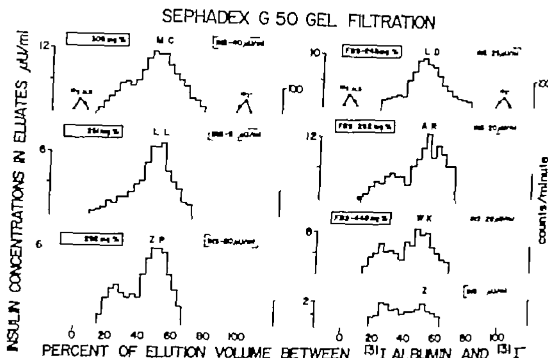


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90 % of the pancreas is required to induce permanent diabetes and in adult dogs with spontaneous diabetes pancreatic insulin content was shown to average less than 4 % of normal (Wrenshall et al., 1954) In contrast in human maturity-onset diabetes, unlike in juvenile diabetics the average value for pancreatic insulin content w shown to vary over a wide range from very low to above the average value for non-diabetic subjects, to average about 50 % that of the non-diabetic and to show little tendency to decrease during the course of the disease (Wrenshall et al 1952). But these studies gave no information on the insulin-secretory capacity of the pancreases which contained normal or even supernormal amounts of insulin. That plasma insulin levels in diabetics are deficient relative to their plasma glucose levels is evident by definition, diabetes is state in which the pancreas is unable to secrete sufficient insulin to render subject euglycemic

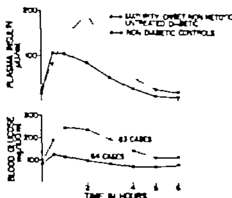


Fig 17
Blood glucose and plasma insulin concentrations during 100 gm oral glucose tolerance test in diabetic and non-diabetic subjects (Reproduced from Berson nd Yalow Proc 3rd Asia and Oceania Congress of Endocrinology Jan 2-6 1967 pp 15-36)

The question of *absolut* versus *relativ* insulin deficiency in the mild maturity-onset diabetic could be answered only by direct measurement. The first studies to which radioimmunoassay was applied (Yalow and Berson, 1960 a, Yalow and Berson, 1960 b) were designed to answer this question W found that in the mild maturity-onset diabetic the secretion of insulin in response to oral glucose loading is initially delayed so that at 30 minutes plasma insulin is lower than in controls but that plasma insulin continues to rise, reaching values by hours or so which are significantly higher than those ever achieved by controls during the glucose tolerance test (Fig. 17). (Yalow and Berson, 1960 a, Yalow and Berson, 1960 b Yalow et al 1965). The delay in secretion was interpreted as due either to a higher glucose concentration threshold for stimulation of insulin secretion or to an inability to produce or mutually to secrete insulin at sufficiently rapid rate (Yalow and Berson, 1960 a, Yalow and Berson, 1960 b). The late high insulin values were attributed to the stimulus of persistent hyperglycemia. It was further suggested that the persistence of hyperglycemia in the presence of adequate insulin-levels was interpretable a insulin insensitivity due to abnormal tissue responses, abnormal insulin molecules or insulin antagonists (Yalow and Berson, 1960 a Yalow and Berson 1960 b). Both of these early observations, i.e the delayed deficient insulin response to glucose and the delayed, deficient glucose utilization in the presence of adequate insulin have been the subject of countless investigations and controversies with respect to fact and interpretation for the past 15 years – and the answers are not yet finalized.

There is fairly general agreement that sluggish or delayed insuli secretion in response to glucose is found in mild diabetes (Yalow and Berson, 1960 a Yalow and Berson 1960 b Perley and Kipnis, 1966 a, Seltzer et al. 1967 Cerasi and Luft 1967 Simpson, et al. 1968). The impaired insulin responsiveness of this group is even more evident following an intravenous bolus injection of glucose than after an oral glucose tolerance test (Perley and Kipnis 1966 a, Seltzer et al.,

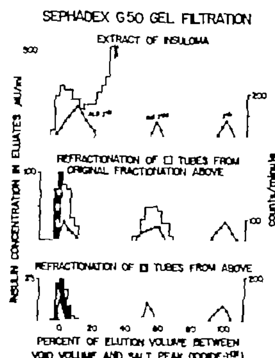


Fig 16 a
Immunoreactive insulin concentrations in Sephadex G-50 gel eluates of a saline extract of an insulinoma (top) and in gel eluates of repeatedly refractionated tubes (stipples) from big big insulin peaks (middle and bottom)

3 Insulin secretion

Since the first application of radioimmunoassay to the measurement of plasma insulin, thousands of research communications have been published dealing with the processes of insulin secretion by the β cells and with the regulation of the insulin-dependent peripheral utilization of metabolic fuels This field has previously been extensively reviewed in several publications In particular the reader is referred to a general review from our laboratory covering investigations through 1968 (Berson and Yalow 1970 b) one by I Arthur Mirsky (1974) completed in 1972 and the review by Cerasi and Luft (1976) in this volume

It is more than 50 years since Banting and Best isolated insulin and demonstrated that this hormone alleviates the similar metabolic derangements of both the depancreatized dog and the diabetic human From their classic work it seemed reasonable to conclude that diabetes in man is attributable to an absolute deficiency of insulin production and/or release by the diabetic pancreas One should however consider that in fact in most animal species experimental removal of more than

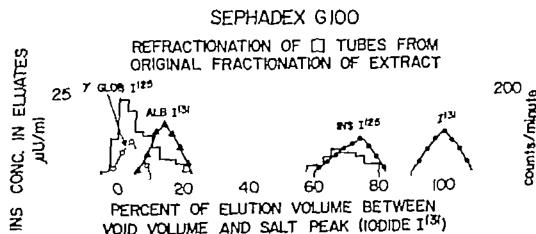


Fig 16 b
Refractionated tube on Sephadex G-100 (stippled) from big big insulin peak of insulinoma extract (A top) (Reproduced from Yalow and Berson 1973)

90 % of the pancreas is required to induce permanent diabetes and in adult dogs with spontaneous diabetes pancreatic insulin content was shown to average less than 4 % of normal (Wrenshall et al 1954) In contrast in human maturity-onset diabetes, unlike in juvenile diabetics, the average value for pancreatic insulin content was shown to vary over a wide range from very low to above the average value for non-diabetic subjects, to average about 50 % that of the non-diabetic and to show little tendency to decrease during the course of the disease (Wrenshall et al., 1952). But these studies gave no information on the insulin-secretory capacity of the pancreases which contained normal or even supernormal amounts of insulin That plasma insulin levels in diabetics are deficient relative to their plasma glucose levels is evident by definition diabetes is a state in which the pancreas is unable to secrete sufficient insulin to render a subject euglycemic

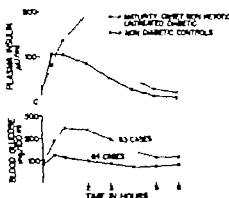


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1967) However there have been several studies demonstrating that intravenous tolbutamide provokes normal insulin release in this group (Perley and Kipnis 1966 a Varsano-Aharon et al 1970 Vague et al 1974) and even the insulin response to glucose can be returned towards normal by pretreatment with theophilline (Cerasi and Luft 1969) or growth hormone (Luft et al 1969) or by simultaneous administration of glucagon (Simpson et al 1968) In mild diabetes normal insulin responses to secretin (Kahil et al 1970) and to isoproterenol (Robertson and Porte 1973) have also been observed There is no consensus also whether there is increased (Berger and Vongaraya, 1966) or diminished (Floyd et al 1970) responsiveness to protein feeding or intravenous amino acids in weight matched mild diabetics compared to normals

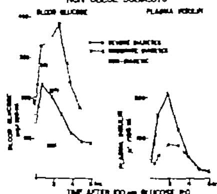
These studies taken together suggest that early mild diabetes is not associated with a generalized defect in insulin secretion but rather with an insensitivity of the insulin releasing mechanism to glucose and perhaps to amino acids

The mechanisms by which changes in or the absolute level of plasma glucose or other secretogogues stimulated insulin secretion are still within the realm of conjecture Whether or not there is convergence of action of all insulin secretogogues through a common path is of clinical as well as of theoretical interest. The diminished responsiveness to a glycemic stimulus and a normal responsiveness to tolbutamide in mild diabetics suggest that there are alternate pathways

Secretion of insulin by the β cell is now known to be effected not only by increases in blood sugar but also by other nutrients by neuronal influences and by a variety of gastrointestinal and perhaps other hormones The pathway for stimulation of insulin secretion appears not to be a unique one – glucose and at least some other agents appear not to follow a common secretory pathway Furthermore since responsiveness of the mild diabetic to many secretogogues other than glucose appears to be normal It is conceptually possible to by-pass the glucose pathway promote normal insulin release and thus effectively treat the disease if it were limited solely to a pancreatic deficit manifested as diminished insulin responsiveness to a glycemic stimulus

Let us now turn to the question as to whether or not there is a peripheral defect in glucose utilization In our first studies on the radioimmunoassay of plasma insulin we found that in mild adult diabetes an oral glucose load is frequently followed by a deficient early insulin response but a greater than normal integrated insulin response because of the failure of the blood sugar to respond appropriately to adequate levels of circulating insulin (Yalow and Berson 1960 a Yalow and Berson 1960 b) This failure we attributed to insensitivity to insulin The question of the insulin intensitivity of diabetes has remained controversial until this time – controversial with respect to fact and/or to interpretation.

Our observations were first questioned by Karam et al (1963) who found that only in obese diabetics was there a high plasma insulin response to glucose loading an observation with which some other workers agree (Floyd et al 1968 Bagdade et al 1968) However many other investigators have confirmed our findings (Yalow et al 1965) that even in non-obese diabetics delayed insulin secretory response is followed by late high plasma insulin concentrations (Perley and Kipnis 1966 a Seltzer et al 1967 Berger and Vongaraya, 1966 Reaven and Miller 1968 Danowski et al 1967 Buchanan and McKiddie 1967). Perhaps the discrepancy is due in part to the type of case material studied When we separated the frankly diabetic group into those with mild to moderate impairment and those with severe impairment of glucose tolerance we used as an arbitrary dividing line a blood sugar concentration of 300 mg per 100 ml at the two hour point in a



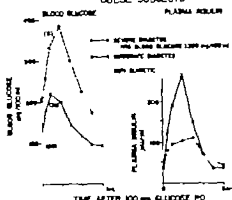


Fig 18
Blood glucos and plasma insulin concentrations in non-diabetic subjects and those with moderat and severe diabet The differentiation between th diabetic groups was on the basis solely of blood blucose thas with 2 hou blood glucose greater than 300 mg/ml were considerede severe A Non-obes s bjects B Obese subjects (Reproduced from Yalow et al 1965)

100 g glucose tolerance test. The group with very poor tolerance in both obese and non-obese categories exhibited greatly dimi nished insulin secretory capacity (Fig. 18) I contrast, those with mild or moderate impairment of glucose tolerance showed greater than normal integrated insulin levels the levels of the obese being somewhat greater than that of the non-obese (Yalow et al 1965)

Several workers whose observations are agreement with ours (Perley and Kipnis 1966 a, Seltzer et al. 1967) disagree with our interpretation. Since the insulin/glucose ratio (Perley and Kipnis 1966 a) or the ratio be tween the increment in plasma insulin and the increment in blood glucose the so-called insulinogenic index (Seltzer et al 1967) is lower in non-obese diabetic patients than in controls these investigators concluded that diabetes is sociated with reduced insulin secretory capacity for any glycemic stimulus and not with reduced sensitivity to insulin. They failed to take into account the fact that in addition, the blood glucose does not respond to the higher plasma insulin levels which would be effective in the non-diabetic Thus it would appear that in diabetics, non-obese or obese factors than plasma insulin per se are involved in disposal of a glucose load

Failure of the blood sugar to respond appropriately to seemingly adequate levels of plasma insulin is not restricted to diabetes per se. There is considerable evidence for the xistence of insulin hyporesponsiveness and of hyperinsulinism in obesity in experimental animals (Christophe et al. 1959) and man (Rabinowitz and Zierler 1962 Karam et al. 1963 Yalow et al. 1965) Diminished responsiveness to plasma insulin can be induced even in normal non-obese subjects by prolonged fasting. Thus starvation diabetes" the impaired glucose tolerance ex hibited by normal subjects after a prolonged fast, is associated with plasma insulin levels greater than those observed after an overnight fast (Unger et al., 1963 Berson et al 1964 Berson and Yalow 1965 Yalow et al. 1965). The blood glucose and insulin

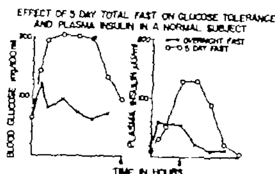


Fig 19
Blood glucose and plasma insulin concentrations during standard oral glucose tolerance tests in a normal subject after an overnight fast and after five days of total fasting (Reproduced from Berson and Yalow 1965)

levels in starvation diabetes resemble those in mild diabetes (Fig 19)

Failure to respond appropriately to elevated plasma insulin levels can also be induced by hypersecretion or by excessive administration of adrenal glucocorticoids (Klink and Estrich 1964 Perley and Kipnis 1966 b) of thyroid hormone (Yalow and Berson 1960 b Klink and Estrich 1964) or of growth hormone (Randle 1954 Yalow and Berson 1960 b Grodsky and Forsham 1960 Cerasi and Luft, 1964 Beck et al 1965) However there is a probably now general but not complete agreement that excesses of these hormones are not to be implicated as causative factors of idiopathic diabetes in man. Nevertheless it was of interest to evaluate whether or not *physiologic* increases in endogenous growth hormone can induce a temporary state of glucose intolerance (Yalow et al 1969) Kipnis (1965) had shown that following the intravenous administration of growth hormone one to two hours are required for impaired glucose tolerance to become definitely evident We reasoned that since plasma growth hormone generally reaches its peak at about *four to five hours in the rebound phase* after oral glucose administration an impairment of glucose tolerance might be observed if a repeat glucose tolerance test (GTT) were performed six hours after the first whereas if the repeat GTT were performed after only three or four hours the insulin insensitivity induced subsequent to the peaking of the endogenous growth hormone might be averted. These predictions were borne out by experimental observations (Yalow et al. 1969). Seven subjects with a peak growth hormone response in excess of 10 ng/ml late in a GTT had during a second GTT test six hours after the first plasma glucose and insulin concentrations resembling those of patients with chemical diabetes (Fig 20 a) In two patients with absent growth hormone responses to provocative stimuli there was no significant

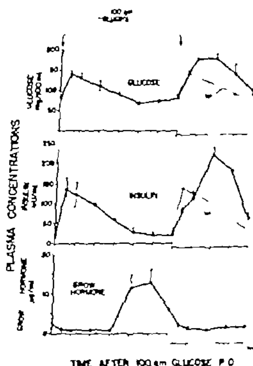


Fig 20 a
Plasma glucose insulin and growth hormone concentrations during oral glucose tolerance test carried out at about 8.30 A M and again six hours later in seven subjects showing a [illegible]
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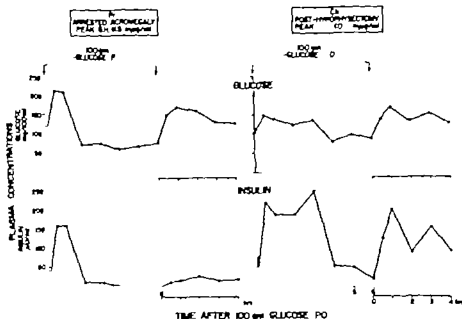
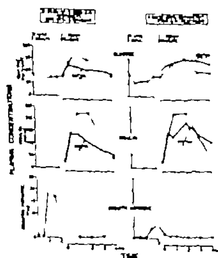


Fig 20 b
Conditions as I figure 20 In two patient th consist ntly absent growth hormon response to provocative stimuli (Reproduced from Yalow et al 1969)



impairment of glucose tolerance during the second GTT and the integrated insulin output during the second test was diminished (Fig. 20 b) Furthermore impaired glucose toler ance and enhanced insulin secretory responses were observed in three good growth hormone responders but not in one poor

Fig 20
Plasma glucose and growth hormone concentrations during insulin toleranc test and plasma glucose insulin and growth hormone concentration during subsequent glucose tolerance tests in three good growth hormone esponders and in one poor responder (peak growth hormone concentration was 6.2 ng/ml during hypoglycemia) Curves marked control GTT were obtained in th same subjects on another day in the fasting state ithout a preceding injections of insulin (Reproduced from Yalow et al 1969).

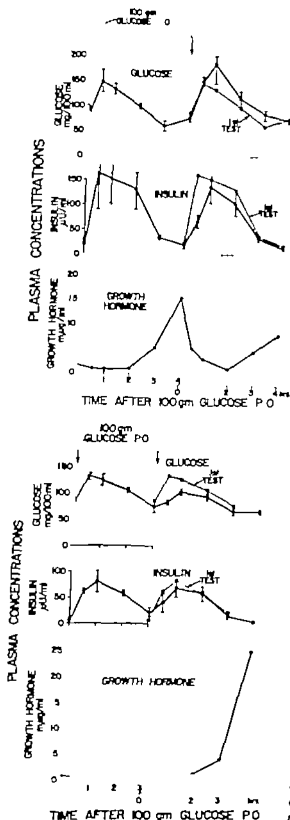


Fig 21
Plasma glucose insulin and growth hormone concentrations during oral glucose tolerance tests carried out at about 8 30 A.M and again four hours later (three subjects) (a) or three hours later (three subjects) (b) (Reproduced from Yalow et al 1969)

growth hormone responder two hours following insulin-induced hypoglycemia (Fig 20 c) Administration of a second glucose load four hours after the first resulted in only a slight impariment of glucose tolerance with no augmentation of insulin secretion (Fig. 21 a) When the second load was given three hours after the first, the rise in growth hormone was prevented and the glucose tolerance improved in spite of a delay in the initial rise of the plasma insulin concentration and a smaller total integrated insulin secretory response (Fig. 21 b).

These experiments suggest an almost constant association of good growth hormone responses with subsequent impairment of glucose tolerance and of poor growth hormone responses with subsequent unimpaired glucose tolerance and are therefore consistent with a cause and effect relationship. Consideration must be given to the possible role of other counter-regulatory mechanisms subsequent to a falling blood sugar The most crucial test would be similar studies in patient with proven isolated growth hormone deficiency but we have not had the opportunity to study such patients.

We also considered whether the worsening of the glucose tolerance in the six hour test might be related to so-called afternoon diabetes (Bowen and Reeves 1967) Therefore in control subjects glucose tolerance tests were performed at 8 30 A M and on another day at 1 30 P M each test being performed after a 14 hour fast Under these circumstances the afternoon test revealed a lower integrated plasma glucose curve than on the morning test so that the worsening of glucose tolerance on the second test given at six hours could not be attributed to diurnal

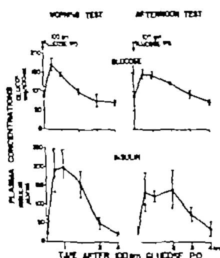


Fig 22
Plasma glucose and insulin concentrations during oral glucose tolerance tests performed in the fasting state at 8.30 A.M after a fourteen-hour fast (left) and on another day at 1:30 P.M after a fourteen-hour fast (right). (Reproduced from Yalow et al 1969)

variability (Fig. 22) In fact the apparent improvement in insulin sensitivity we observed, if real, might be due to the ACTH and steroid levels being lower in the afternoon than in the morning.

Thus an afternoon glucose tolerance performed six to seven hours after breakfast may result in apparent chemical diabetes in otherwise normal subjects. Furthermore it must be remembered that stress, psychologic and physical, can also serve as a growth hormone secretogogue so that occasional abnormalities of glucose tolerance secondary to unsuspected spurts of GH may well be observed in otherwise normal subjects. Variable GH values alone could perhaps account for the observations by McDonald et al. (1965) who studied the reproducibility of blood glucose responses of 334 male volunteers who were known not to be diabetic

Each subject had six 100 g oral blucose tolerance tests administered over a one year period. The average blood glucose levels for the total group were found to remain stable and some individuals had similar blood glucose levels in all six tests However some individual responses varied greatly from low normal to frankly diabetic in one or more of the tests Our own experience in repeat glucose tolerance tests over more than a 10 year period in laboratory personnel known to be non-diabetic confirms the findings of McDonald et al (1965).

Considering the diversity of factors that influence response of plasma glucose to plasma insulin levels it is not surprising that occasional abnormalities of glucose tolerance are observed in non-diabetic subjects However even when the glucose tolerance tests are normal, ery great differences in insulin sensitivity among individuals are found so that the standard deviation of plasma insulin concentrations during an oral glucose tolerance test may be almost equal to the mean value (Fig. 23) We believe that the preponderance of evidence suggests that plasma insulin concentrations during an oral GTT follow the changes in blood glucose

Fifteen years ago when we postulated the concept of insulin insensitivity in diabetes, we considered whether it was due to insulin antagonists in plasma, an abnormal insulin, or some abnormality of the tissue response At present there is little or no support for a plasma factor which serves as an insulin antagonist or for an abnormal insulin. The question of abnormalities of tissue responsiveness has now translated into inquiring into the occurrence of abnormalities in tissue recept or sites for insulin. Since diminished insulin responsiveness may be associated with diabetes, obesity excess of several hormones fasting, etc. is there a pattern of alteration in insulin receptor sites common to all? Is diminished insulin responsiveness in diabetes associated with a decrease in insulin binding to specific receptors as has been postulated to

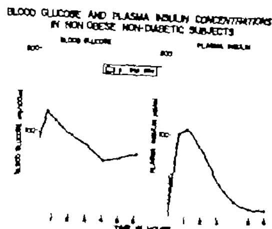


Fig 23
Mean blood glucose and plasma insulin concentrations in 38 non-obese non-diabetic subjects The shaded area corresponds to ± 1 standard deviation from the mean The percentage standard deviation of the insulin concentrations averages six fold greater than the percentage standard deviation of the glucose levels

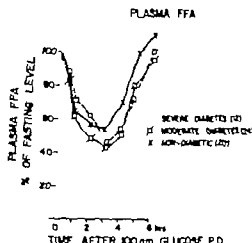


Fig 24
Plasma FFA concentrations during six hour oral glucose tolerance tests as per cent of the fasting level in patients with severe diabetes and moderate diabetes and in non-diabetic subjects Blood glucose and plasma insulin concentrations for these subjects are shown in Fig 18 Obese and non-obese patients were pooled for this figure (Reproduced from Yalow et al 1965)

occur in obesity (Archer et al 1973) and glucocorticoid excess (Kahn et al 1973)?

In fact can one expect insulin receptors from all tissues in the same subject to behave similarly? Consider the following – the sharp reduction of plasma FFA in obese and diabetic subjects whose blood sugar remains elevated in the face of normal or elevated plasma insulin concentrations indicates that in contrast to glucose utilization in these patients the *lipolytic* process is not insulin – resistant (Yalow et al 1965) Even in severe non-ketotic diabetics who show only slow and relatively small rises in plasma insulin the fall in FFA is as great as in non-diabetic and moderate diabetic subjects following an oral glucose load (Fig. 18 Fig. 24) Thus in spite of evidently poor *glucose* utilization only a small amount of insulin is required to effect a reduction in plasma FFA Further evidence concerning dissociation of insulin sensitivity in different insulin-dependent tissues was the observation of Mirsky (1963) more than a decade ago that is possible to lower plasma FFA in normal and alloxan diabetic dogs with amounts of insulin too small to affect the blood sugar In addition it is evident that the ability to use glucose for fat synthesis must be quite intact in obese diabetics in spite of generally impaired glucose utilization Yet insulin is required for below some critical insulin concentration uncontrolled fat catabolism ketosis and failure of lipogenesis do take place. These considerations suggest therefore that it is not unreasonable to expect that different target cells and their receptor sites in the same subject at the same time may show marked differences in insulin sensitivity Thus there is much still to be learned as to whether receptor assays will provide significant answers to the enigmatic question as to the nature of the fundamental defect in diabetes

Some other aspects of immunology related to the pathogenesis of diabetes

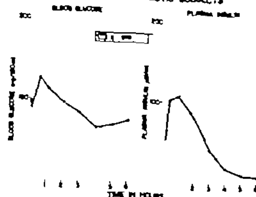
In this section we will consider some observations which do not involve radioimmunologic methodology but which do relate to current ideas in the immunology and pathogenesis of diabetes

1 Maturity-onset and juvenile-onset diabetes – the same or different diseases?

Perhaps one of the most interesting papers considering this problem recently is that of Tatteral and Fajans (1975) who suggest that there is difference in the inheritance of diabetes in patients diagnosed before 25 years of age with either classical juvenile-onset diabetes (JODY) and those who resemble more the maturity-onset type (MODY). The former patients are those whose disease is characterized by abrupt-onset, severe symptoms and tendency to ketoacidosis the latter are those with milder form of diabetes, without symptoms of ketonuria and with fasting hyperglycemia which can be corrected without insulin. I the families of the MODY 85 % of the propositi had diabetic parent, 53 % of the siblings had mild asymptomatic diabetes and there was vertical transmission of diabetes through 3 generations in 46 % of the families. In contrast in the families of the JODY group only 4 % of the propositi had a diabetic parent, three generation inheritance was found in only 6 % 8 % of the siblings were insulin-requiring and 3 % had abnormal glucose tolerance tests Unfortunately the studies appear to be too recent to determine whether there was a difference in the incidence of vascular complications in the two groups and the authors did not indicate that such studies were in progress.

These results are consistent with the possibility that in some cases at least juvenile-onset diabetes may not be genetic disease but may arise as a consequence of acute pancreatic destruction of known or unknown origin This possibility is suggested also by the earlier paper of Tattersal and Pyke (1972) on the incidence of diabetes in 96 pairs of identical twins Of these 65 were concordant (both diabetic) and 31 were discordant (one twin diabetic) Of the 37 pairs in which the index twin was diagnosed after the age of 40 all but 3 were concordant and 56 of 68 individual members of this set did not require insulin. However of the 59 pairs in which the index twin developed diabetes before 40 only half were concordant and over 90 % of all members of this group required insulin.

In subsequent report on the same group of patients the prevalence and features of diabetic retinopathy were examined in those twins one of whom had been diabetic for at least 15 years (Pyke and Tattersal 1973). In the 13 concordant pairs retinopathy was present in 23 of the 26 patients and 7 were blind or partially sighted In the 10 discordant pairs retinopathy reported to be conspicuously mild, was present in only 5 of the patients and none wer blind. There were no retinal abnormalities in the non-diabetic members of the discordant pairs. I both concordant and discordant groups the mean age at diagnosis was about 26 years and the mean duration of diabetes was about 22 years and all but 3 patients were insulin-dependent. It is interesting to note that diabetic retinopathy appeared to be more severe in the concordant group although there was no evidence for differences among the groups with respect to diabetic control Although this group is



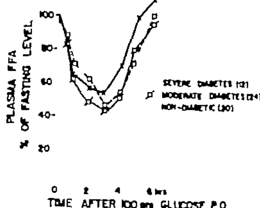


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Some other aspects of immunology related to the pathogenesis of diabetes

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These results are consistent with the possibility that in some cases at least juvenile-onset diabetes may not be a genetic disease but may arise as consequence of acute pancreatic destruction of known or unknown origin. This possibility is suggested also by the earlier paper of Tattersal and Pyke (1972) on the incidence of diabetes in 96 pairs of identical twins. Of these 65 were concordant (both diabetic) and 31 were discordant (one twin diabetic) Of the 37 pairs in which the index twin was diagnosed after the age of 40 all but 3 were concordant and 56 of 68 individual members of this set did not require insulin. However of the 59 pairs n which the index twin developed diabetes before 40 only half were concordant and over 90 % of all members of this group required insulin.

In a subsequent report on the same group of patients the prevalence and features of diabetic retinopathy were examined in those twins one of whom had been diabetic for at least 15 years (Pyke and Tattersal, 1973). I the 13 concordant pairs retinopathy was present in 23 of the 26 patients and 7 were blind or partially sighted. In the 10 discordant pairs retinopathy reported to be conspicuously mild, was present in only 5 of the patients and none wer blind. There were no retinal abnormalities in the non-diabetic members of the discordant pairs. In both concordant and discordant groups the mean age at diagnosis was about 26 years and the mean duration of diabetes was about 22 years and all but 3 patients were insulin-dependent. It is interesting t note that diabetic retinopathy appeared to be more severe in the concordant group although there was no evidence for differences among the groups with respect to diabetic control Although this group is

clearly too small to permit one to arrive at definitive conclusions the evidence is suggestive that diabetic retinopathy is more frequent and severe when there is strong evidence for genetic diabetes and is not as prominent when the genetic factor is less obvious

2. Some considerations of a possible viral etiology for acute juvenile-onset diabetes

The reports of Tattersal and coworkers just discussed are consistent with the possibility that an acute pancreatic destructive process may be implicated in some cases of juvenile onset diabetes Viral infections could perhaps be the causative factor in some of these cases For more than a century since the Norwegian physician J Stang, first reported in 1864 that diabetes developed in one of his patients after a mumps infection there have been scattered reports of temporal associations between a variety of viral infections and diabetes (see Maugh 1975 for review) Although in earlier years mumps virus was reported to be the one associated most consistently with diabetes recently Coxsackie B4 virus has been indicted as the culprit (Gamble et al 1973) In one such study Gamble et al (1969) reported that of 64 insulin-dependent diabetics seen within three months of onset of diabetes 66 % had Coxsackie B4 virus antibody titres of 1 20 or greater compared with 40 % of non-diabetic controls who had no history of active or recent infection and that the incidence of antibody to other Coxsackie B types did not differ in the diabetics from that of the control group In another group of patients studied over a three year period from 1970 to 1973 Gamble et al (1973) reported that 70 % of 162 insulin-dependent diabetic subjects of recent onset had antibody to Coxsackie B4 virus compared to 58 % of a control group of 319 These authors considered these differences to be significant However in view of the potential role of geographical distribution of virus infection it is of interest that only 22 of the controls came from the same geographical location as the diabetics – of these only 14 or 64 % had antibody to virus However if 13 instead of 14 diabetics from the same region as the control group had had antibody the incidence of antibody in the control and diabetic groups (58 % and 59 % respectively) would have been identical The statistical basis on which Gamble and associates (1973) appear to implicate Coxsackie B4 virus in juvenile-onset diabetes is so weak that it is not surprising that their findings have not been supported by other investigators Thus according to a recent review (Maugh 1975) J.A Weaver et al at Royal Victoria Hospital in Belfast Ireland P H Bennett et al of the Epidemiology and Field Studies Branch of the National Institute of Arthritis Metabolism and Digestive Diseases Phoenix Arizona who studied an epidemic of Coxsackie B4 infections that struck the isolated Pribiloff Islands during the winter of 1967 1968 and J C Hierholzer et al at the Center for Disease Control Atlanta who studied an outbreak of Coxsackie B3 and B4 virus in a children s home in 1968 all reported no link between the B4 virus and diabetes

Clearly the numbers involved in all these studies are hardly adequate for statistical analysis However there is no doubt that at present there is no stronger evidence implicating Coxsackie B4 virus as a significant factor in the etiology of juvenile-onet diabetes than there is denying such a linkage

3 Histocompatibility antigens and diabetes

Recently considerable interest has been generated in the search for association between antigens of the major human transplantation or histocompatibility antigen system (HL A system) and susceptibility to a variety of neoplastic autoimmune and infectious diseases in man (McDevitt and Bodmer 197? 1974) In some cases the association would appear to be proven Thus the HL-A antigen

W27 was identified in 72 out of 75 patients with classical ankylosing spondylitis 31 out of 60 first degree relatives and only 3 out of 75 controls (Brewerton et al. 1973) The same antigen appears to be also implicated in Reiter s disease and in acute anterior uveitis. In both conditions the incidence was more than 7 fold as high in the affected patients than in the controls. (McDevitt and Bodmer 1974)

To our knowledge there have been four studies of the HL-A system and diabetes. Finkelstein et al. (1972), who compared the haplotypes of 44 insulin-dependent juvenile-onset diabetics with normal genotypic data obtained from the IV International Histocompatibility Workshop in 1970 reported no significant differences between the groups The results of the other three papers are tabulated below

	HL A8 (C)	HL A8 (D)	W 15 (C)	W 15 (D)	HL A11 (C)	HL A11 (D)
Singal and Blajchman (1973)	20 %	24 %	10 %	36 %	12 %	18 %
Nerup et al. (1974 a)	24	42	17	35		
Cudworth and Woodrow (1975)	32	54	12	18	16	

Although these results might be considered as suggesting an increased frequency of the HL-A8 and W15 antigens in insulin-dependent diabetes, it is apparent that there are marked differences in the frequencies observed by the different investigators in both control and diabetic groups. Do these differences simply arise from methodologic variations among the laboratories perhaps due to the use of different antisera or I the frequency of the HL-A antigens so variable from group to group that these differences have no real statistical significance? For instance the relative frequencies of the HL-A11 antigen in the control and diabetic groups of Singal and Blajchman (1973) are identical with that for W15 antigen as reported by Cudworth and Woodrow (1975) Since the latter interpret their W15 data as demonstrating an association between the antigen and susceptibility to insulin-dependent diabetes then the data of Singal and Blajchman (1973) should be interpretable as *showing the same* relation for the HL-A11 antigen. However the Cudworth-Woodrow data for the HL-A11 antigen would equally as well be interpretable as demonstrating that the antigen is associated with protection against the diabetic state. In fact none of these data are convincing as are the data for the W27 antigen It would appear that from the reports so far available it is premature to attribute great significance to the slight differences occasionally observed in the frequency of the histocompatibility antigens between the diabetic and non-diabetic group

4 Cell-mediated immunity in diabetes

The relation between diabetes and autoimmune processes has excited some interest in recent years. A discussed earlier (see Sec II 3) there is no convincing evidence that endogenous insulin can be implicated in the development of circulating insulin-binding antibodies since these are never found without prior exposure to exogenou insulin.

Recently several in vitro tests have been employed to study cell-mediated immunologic function. The method of antigen-induced inhibition of the migration of leucocytes (LMT) (Soborg and Bendixen, 1967 Soborg, 1968) has been used to demonstrate inhibition of diabetic leucocytes in the presence of antigens derived from the pig (Nerup et al., 1971) fetal calf (Nerup et al. 1973 Nerup et al., 1974) or human pancreas (MacCuish et al. 1974 a). According to MacCuish et al (1974 a) the effect was reported to be most

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Conclusions

Radioimmunologic methods for detection of antibodies and measurement of hormone levels are now well-established and in use around the world in countries large and small developed and underdeveloped. They have led to an enormous increase in our understanding of the factors governing the secretion of insulin and the consequent regulation of the utilization of metabolic fuels Replacing conjecture with quantification has increased our understanding of the complex interrelationships among the multiplicity of nutrient, hormonal neurohumoral, toxicologic and physical influences which modulate the role of the glycemic stimulus as in insulin secretogogue. In turn the effectiveness of insulin in promoting glucose utilization has been shown to be modulated by a variety of known and unknown hormonal and other factors There is still much to be learned in these areas. There has been no systematic study of insulin release in non-diabetic and early mild diabetic subjects obese and non-obese during an average day with normal meals and normal activity Such a study should include measurement of plasma concentrations of blood glucose amino acids and free fatty acids as well as simultaneous measurements of hormones other than insulin which are considered to be insulin secretogogues or to be involved in the regulation of blood glucose These would include among others, glucagon, ACTH glucocorticoids growth hormone somatomedins and the gastrointestinal hormones. It would be of interest also to study the changes in the hormonal substrate interrelationships in the diabetic who is undergoing significant weight loss or gain or who is being treated with one of the oral hypoglycemic agents for an extended period.

Unanswered as yet is the really important question. Are diabetic retinopathy neuropathy and nephropathy concomitants of genetic diabetes mellitus or are they complications arising from relative or absolute hypoinsulism and the metabolic derangements consequent thereto Solution of this problem is required to gain fundamental insight into the nature and management of diabetes, but the design of experimental studies to give a definitive answer is difficult and challenging.

It is commonly accepted that genetic factors are important in the etiology of idiopathic diabetes mellitus (Rimoin, 1967) Yet the mode of inheritance and the nature of the fundamental defect( ) are not well defined Inherent in current research efforts directed at the development of an artificial beta cell with its computerized glucose sensor and insulin injector or perfection of beta cell or pancreatic transplant techniques is the implicit assumption that the full manifestations of genetic diabetes are derived simply from pancreatic failure However attempts to establish a direct causal relationship between the severity of the insulin deficiency and the metabolic consequences thereof on the one hand and the various types of vascular degeneration found in diabetes, on the other have not met with success (see Knowles, 1970 for brief review) By this time one might have expected such data to be available since the use of hypoglycemic agents over the past two decades has undoubtedly resulted in better normalization of blood sugar levels throughout the day for the more than million diabetic patients treated with these agents. However such evidence has not been forthcoming. The University Group Diabetes Program UGDP) ios the largest controlled clinical trial of oral hypoglycemic agents to date (UGDP 1970 a, b 1971 a, b 1975) While the results of this study have been controversial and many diabetologists contest the conclusion that tolbutamide and, more particularly phenoformin might be implicated in an increased risk of cardiovascular complications and mortality therefrom, none would disagree that the presumed im-

prominent in recently diagnosed juvenile onset diabetics Using LMT there has been no evidence for inhibition by beef or pork insulins (Nerup et al 1971 1973 1974 MacCuish et al 1974 a) but inhibition has been reported using human and rat liver mitochondria (Richens et al 1973 1974) Since inhibition of leucocyte migration by liver mitochondria is present in Hashimoto s thyroiditis (Brostoff 1970 Calder et al 1972) pernicious anemia (Brostoff 1970 Coldstone et al 1973) and primary biliary cirrhosis (Brostoff 1970) the specificity of this reaction can well be questioned It would be of interest to extend these studies to patients with acute or chronic pancreatitis with or without diabetes to determine if in cases of destruction of the exocrine pancreas there is sensitization to pancreatic antigens as well

Mitogen – or antigen induced transformation of lymphocyte to blast cells are alternative in vitro tests of cellular immune function (Bloom 1971) Since diabetics manifest increased sensitivity to infection consideration has been given to their having a defect in their immune-response system Lymphocyte transformation to blast cells induced by the mitogen phytohemagglutinin (PHA) is employed to test for such a defect (Roitt et al 1969 Bloom 1971) Several studies employing this PHA have demonstrated that the transformation responses are normal in well-controlled diabetics although depressed in poorly controlled diabetics (Brody and Merlie 1970 Ragab et al 1972 MacCuish et al 1974 b 1975) Of particular interest are the recent studies of MacCuish et al (1975) who employed insulin-induced transformation of lymphocytes and noted the blastogenic effect not only in a group of established diabetics all of whom had been insulin treated for months or years and who therefore undoubtedly had circulating antibodies to insulin but also in six of ten newly diagnosed patients four of whom had never been given insulin This very interesting and unexpected finding that some diabetics have an insulin-sensitized lymphocyte population before exposure to exogenous insulin requires confirmation by studies with a larger group of control subjects with a group of established diabetics with no history of insulin therapy as well as with newly discovered juvenile-onset type diabetic subjects. Another unexpected feature of the studies of MacCuish et al. (1975) is their demonstration that the isolated B chain of beef insulin was more potent in stimulating blastogenesis than the isolated A chain and even more potent than insulin itself in some diabetics This is in contrast to the humoral response to insulin where studies seem to suggest that A chain is more prominent in antibody production (Berson and Yalow 1959 Wilson et al. 1962)

This review is too brief for detailed analysis of all aspects of the role of autoimmunity in diabetes Consideration of the studies reported up to now suggest that there is no relation between autoimmunity and non insulin requiring adult-onset type diabetes that there may be a relation in some cases of juvenile-onset type diabetes but that the evidence can at best be considered presumptive until larger groups of diabetic patients and controls are studied with the control group including those without diabetes but with destructive processes in the exocrine pancreas such as acute and chronic pancreatitis or cystic fibrosis

Appendix

I. Preparation and purification of radioiodine labeled insulin

Although ^{131}I (half-life = 8 days) was the nuclide first used for the preparation of radioiodine-labeled insulin ^{125}I (half-life = 60 days) has now become the radioisotope of choice for labeling peptide hormones. Although ^{131}I theoretically has a more than 7-fold advantage in specific activity because of its shorter half-life thi advantage is not practically realizable since isotopically pure ^{131}I is not available so that the pecific activities (disintegration rate/weight of che mical iodine) of ^{125}I and ^{131}I at the time of receipt in the laboratory do not differ significantly

Radioiodine prepared for clinical purposes is generally not suitable as a labeling reagent and special solutions without added unlabeled iodine or reducing agent should be used. The longer half-life of ^{125}I permits use of labeled preparations for an extended period of time generally weeks or even months. Furthermore the low energy of X and gamma radiation from ^{125}I is advantageous since there is high sensitivity for detection of this radiation in the usual well-scintillation counter and the problems of radiation protection are simplified by the low penetration of this radiation. For iodination we hav found minor modification of the chloramine T technique (Hunter and Greenwood 1962) to be most suitable A radioiodine to hormone ratio of 380 μCi ^{125}I per microgram of insulin has been calculated to result in the generally de sired average of one iodine atom/molecule insulin if the iodination efficiency is 100 %. Using the following iodination conditions, about 80-90 % efficiency is generally obtainable (Yalow and Berson 1973 b). To 20μl phosphate bugger (0 25 M pH 7 4) are added successively ^{125}I (380 μCi) insulin (1 μg), 10 μl chloramine T (5 4 mg/ml phosphate buf fer) 20 μl sodium metabisulfite (4.8 mg/ml phosphate buffer) and 20 μl human plasma. The entire iodination procedure should take no more than 15 seconds. Purification can be effected by starch gel electrophoresis Sephadex G 50 gel filtration or adsorption to andelution from a cellulose column. On starch gel electrophoresis both labeled and unlabeled insulins migrate in advance of serum albumin, the more highly iodinated fractions having the more anodal mobility (Fig. 25) (Berson and Yalow 1966 c) The labeled hormone eluted from the first spot immediately in advance of the albumin contains 1 iodine atom per molecule and has been shown to behave immunologically with all our antisera like unlabeled insulin. The labeled insulin in the most anodal spots shows diminished immunoreactivity shorter shelf-life and increased tendency to be damaged on incubation during radioimmunologic or radioimmunoassay procedures. Labeled insulin purified on Sephadex or cellulose columns contains a mixture of lightly and more heavily iodinated fractions and therefore tends to be less stable than the material purified on starch gel.

Over the past few years interest in receptor-hormone interactions and radioreceptor

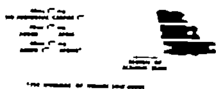


Fig 25
Autoradiograph of starch-gel electrophoresis of I^{125}-ins lin preparations The numbers given for iodine atoms per molecule of insulin indicat the average valu for the preparation they are calculated as the product of the starting ratios and the percentage iodination. (Reproduced from Berson nd Yalow 1966 a)

provement in diabetic control in the group treated with hypoglycemic agents compared to the placebo group did not result in a dramatic decrease in the incidence of the vascular lesions of diabetics Large scale well-designed cooperative studies similar to the UGDP study are certainly necessary to provide the crucial data concerning the re lation between control of diabetes and the metabolic derangements consequent thereto and the other concomitants of the disease

Gazing into a cloudy crystal ball to ask from where one can hope for the most unexpected and important breakthroughs in diabetes in vestigation one can perhaps see a path leading to an increased understanding of the genetic nature of the disease Certainly the studies of Tattersall and Fajans (1975) which suggest a different mode of inheritance of diabetes in young patients who have the classical severe juvenile-onset type diabetes and those with the mild maturity-onset type disease are provocative need more extensi ve verification and should be supplemented by clinical follow-up which evaluates the incidence of the vascular complications of both groups The studies of 96 pairs of iden tical twins (Tattersall and Pyke 1972 and Pyke and Tattershall 1973) are particularly exciting for they suggest that diabetic retinopathy is found most regularly and in a very severe form when genetic diabetes appears to be the culprit and seems to occur less frequently and in less severe form when the genetic element is less clear Where do theories concerning the role of viral or auto-immune mechanisms in the etiology of dia betes fit into these considerations? If in fact the studies of Tattersall and associates are valid then it may well be that the acute pancreatic destruction found in the juvenile onset group is not a marker for the genetic disease Perhaps the next decade may provide answers to the critical question – what is genetic diabetes?

tions are used to take advantage of the possible slight denaturation of such preparations which renders the hormones "foreign" and thereby enhances their antigenicity. Production of antibodies to a small peptide may be difficult unless its antigenicity is enhanced by coupling to a larger protein. Carbodiimide (Goodfriend et al. 1964) or glutaraldehyde (Habeeb and Hiramoto, 1968) are commonly used as the coupling agents.

Since the presence of other immunologic reactions does not interfere with the reaction between labeled antigen and its specific antibody, immunization with several unrelated antigens can be performed simultaneously. For example, peptide hormones such as insulin, hGH, glucagon, ACTH, parathyroid hormone and gastrin could be administered simultaneously since there are no regions of structural homologies among these molecules. Multiple-antigen immunization is advantageous in reducing the number of animals to be immunized and to be bled by a factor equal to the number of antigens used simultaneously. The antibody concentration and the sensitivity and specificity of antibodies to the different antigens appear to be unrelated to each other and to whether or not they are administered simultaneously.

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assays has led some investigators to conclude that labeled hormones suitable for radioimmunoassay may not be suitable for receptor assays (Roth 1973) It has been suggested that at the same ^{125}I insulin ratio the use of stoichiometric concentration of chloramine T affects the distribution of iodine atoms incorporated in the insulin molecule (Gavin et al 1972) The results of our recent reinvestigation of this problem (Schneider et al 1976) suggest that iodination of insulin in aqueous solution at an average of 1 iodine atom per molecule insulin results in the same distribution of iodine atoms dependent only on the average iodine number and independent of the iodination method and that this distribution can be calculated on the basis of a Monte Carlo stimulation It has been calculated theoretically and demonstrated experimentally that for insulin iodinated at an average of 0 8 iodine atoms per molecule approximately 50 % of the radioactivity is incorporated in other than monoiodoinsulin and that the more highly iodinated species do bind to the lymphocyte receptor Since as in radioimmunologic procedures the overiodinated species, while binding to specific receptors are less satisfactory for use as tracers and since receptor assays seldom approach the sensitivity of radioimmunoassays it is probably desirable in receptor assays to prepare labeled peptide hormones at lower specific activities than are generally required for the more sensitive radioimmunoassay

Iodination of other peptide hormones may be performed similarly to that of insulin It is generally desirable to iodinate to an average of less than one iodine atom per molecule The iodination procedure should be rapid to prevent damage to the hormone by the oxidizing agent, and purification should be effected where possible by some system that separates by charge such as electrophoresis or ion-exchange chromatography in order to resolve the more highly iodinated components

Iodination with chloramine T has the advantage that the oxidation takes place at an alkaline pH so that there is no volatilization of radioiodine. An iodination procedure which requires an acid pH should be performed in a closed system to reduce the radiation hazard associated with radioiodine Use of a closed system adds some technical complications and is generally excessively inconvenient for a non-commercial research laboratory

2. Production of antibodies

Antisera suitable for radioimmunoassay of human insulin cannot be readily produced in rabbits since rabbit and human insulins are identical except for the terminal amino acid of the B chain (Smith 1966) In contrast pork and human insulins are highly antigenic in guinea pigs since endogenous guinea pig insulin differs from that of other mammalian insulins in multiple sites (17 amino acids differ from those of human insulin (Smith 1966)) Immunizing doses consist of 5-10 units of commercial pork insulin administered as regular insulin homogenized with an equal volume of Freund s complete adjuvant or as protamine zinc or NPH insulin without adjuvant Maximal titres may be reached after 3 to 5 doses spaced ? to 4 weeks apart The antisera obtained can be used usually at dilutions of 1.50 000 or greater some even at a dilution of 1 500,000 (Fig 11) Concentrations as little as 0.2 to 0 5 microunit insulin per milliliter can readily be assayed with some antisera (Fig. 11)

Human growth hormone (hGH) is highly antigenic both in guinea pigs and rabbits and suitable antisera are readily obtained after 3 immunizing doses of hGH administered in Freund s complete adjuvant as described above

Most peptide hormones are satisfactorily immunogenic in a variety of experimental animals especially when the hormone is administered as an emulsion in Freund s adjuvant For immunization with most hormones commercial or low purity hormonal prepara

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Insulin preparations

1 History

After it had been demonstrated by von Mering and Minkowsky in 1889 that pancreatectomized dogs developed diabetes mellitus, numerous attempts were made to extract from the pancreas an active principle for its treatment (69 115 149 179).

These attempts did not lead to the desired result, but gradually it was realized that the active principle was formed in the islets of Langerhans (155) Therefore de Meyer (117) named the hypothetical substance insulin. It became evident also that this hypothetical insulin was split by the exocrine pancreatic enzymes Accordingly experiments were started on atrophic pancreases and pancreases from calf foetuses which did not exhibit any trypsin action. Taking these factors into consideration Paulesco in Roumania, succeded in obtaining the first active insulin extracts between 1916 and 1921 (110) It is the credit of Banting and Best that, unaware of Paulesco s results, they were able to develop the first active insulin preparations applicable for man in 1921 in Macleod Laboratory in Toronto (18). The discovery was placed at the disposal of the Toronto University whence the Insulin Committee generously made it available against certain guarantees In U.S.A the licence was given to Eli Lilly (1922) in Germany to Farbwerke Hoechst (1923) and in Scandinavia to professor August Krogh, who together with Hagedorn and Kongsted founded Nordisk Insulin Laboratorium.

The first insulin preparations contained appreciable quantities of contaminating pancreatic proteins with little hypoglycemic action (biological activity 8 0 U/mg (106) and were not stable in neutral solution The patients were therefore supplied with tablets or powders which had to be dissolved before the injection. As a result, many patients developed sterile abscesses at the sites of injection.

Following the discovery of insulin, extensive research was started to produce insulin in a pure form, and an intensive effort was made to raise the yield of insulin extraction from the pancreas.

Abel in 1926 (1) showed that insulin could be made to crystallize, the first protein to do so and after Scott and Fisher in 1935 (150) described the crystallization procedure the crystalline state became the most usual form for the technical production of insulin. In the course of time there have been many detailed changes in the preparation of insulin These details vary from one factory to another and have only been published in part.

2. Production

Insulin can be produced synthetically. Since however synthetic insulin of a primary structure like human insulin does not afford clinical advantages above non-synthetic porcine insulin and since the production of synthetic insulins must be expected to remain extremely costly for many years to come (178) insulin for clinical use will continue to be produced from animal pancreases generally from beef pancreases, less frequently from pig pancreases and also from sheep whale and fish pancreases. For many years possible species differences among the insulin were not considered During that period bovine and porcine insulins were extracted and purified together After the species specificity and side effects related to species difference were recognized, several insulin factories separated the production and are now making species specific insulins – the so-called monospecies insulins.

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The production of insulin may be divided into 3 main steps

1 collection and storage of pancreas glands
2 extraction and purification of insulin
3 physical chemical and biological testing of the insulin crystals

Collection of glands

The pancreas is removed at the slaughter house freed of attached fat and immediately placed in containers for deep-freezing to pre vent bacterial and enzymatic decomposition The tightly packed glands are then transport ed deep-frozen to the insulin factories These procedures for collection storage and transport have proved to be of great impor tance in increasing insulin yield and the pro duction of a pure insulin preparation. The yield also varies with the season and with the age and nutritional condition of the animals.

Extraction

When the production of insulin is to be start ed the deep-frozen blocks of pancreas are minced by machine and extracted with acid aqueous ethyl alcohol in which insulin but not *most other tissue proteins is freely soluble*

The acid reaction has the effect of inhibiting the action of the proteolytic enzymes. The acid alcoholic extract is filtered and then neutralized The precipitate is removed by centrifugation and discarded The insulin containing supernatant liquid is then weak

Table 1
Characteristics of insulin preparations
sol = solution susp = suspension A = amorphous C = crystalline a = acid n = neutral P = porcine B = bovine PB = mixed porcine and bovine

		physical state: sol	physical state: susp A C	pH: a	pH: n	species: P	species: B	species: PB	purity: recryst	purity: highly purified	retarding substances mg/100 units	effect (hours): initial	effect (hours): max	effect (hours): final
RAPID	Regular (ALT NORMAL)	+	−									/	3	6
INTERMEDIATE	NPH (isophane)										0 3–0 6 mg protamine	1 /	8	18
	Depot (Horm)										0 6 mg protamine 0 15 mg Zn	1	5	18
	Semilente										0 2–0 25 mg Z	1	5	14
	Lente		A & C								0 2–0 25 mg Zn	1 /	8	26
	Monotard		A & C								0 2–0 25 mg Zn	1 /	8	18
	Depot(Hoechst)										0 4 mg Surfen	1 /	4	16
	KOMB(Hoechst)										0 28 mg Surfen	1	3	14
	Globin insulin										2–4 mg globin 0 3 mg Zn	1 /	5	16
SLOW	ZPI (zinc protamine insulin)										1 25 mg protamine 0 2 mg Zn	3	10	48
	Ultralente										0 2–0 25 mg Zn	3	10	72
	Long insulin		A & C								0 12 mg Surfen	3	8	28
BIPHASIC	Insulard										0 15–0 3 mg protamine	/	5	16
	Rapitard											/	7	24

ly acidified and the alcohol is removed by evaporation under vacuum at low temperature. Alcohol-soluble lipids, which separate out as a result of the diminishing alcohol concentration, are then removed by filtration, and the raw insulin is salted out usually by means of sodium chloride at weak acid reaction. The isolated crude insulin is dissolved in dilute acid, after which the solution is adjusted to the iso-electric point of insulin, approximately pH 5.2. The precipitate produced consisting mainly of insulin is redissolved and crystallized. Crystallization is effected by precipitating the insulin as a zinc compound from an aqueous insulin solution at reactions which are slightly above the iso-electric point. The zinc content of the crystalline insulin is between 0.4 and 0.8 % of the insulin. Other purifying procedures and/or one or several recrystallizations then follow varying from factory to factory whereupon the injection preparations can be produced

Over the years the insulin yield has increased from about 50 to 150 mg insulin per kg pork pancreas. In the case of beef pancreas the yield is about 100 mg per kg. Higher yields are obtained from calf-pancreas. For human pancreas the yield is less, depending upon the initial material, about 50 mg insulin per kg.

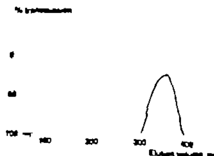


Fig. 1
Gel chromatography of highly purified porcine insulin on Sephadex G-50 in 1 M acetic acid

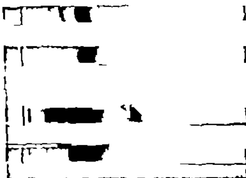


Fig. 2
Disc electrophoresis of 100 µg and 500 µg crystalline porcine insulin and highly purified porcine insulin respectively in polyacrylamide gel

Testing

The first insulin preparations commercially available consisted mainly of contaminated substances (106), but the insulin now available is relatively pure. The most recent fourth international standard insulin preparation is from repeatedly crystallized insulin and has a biological activity of 24 units/mg (4.8 % water). However even repeatedly recrystallized insulin is not so pure that it can be regarded as a homogeneous, well-defined substance. To characterize the purity of an insulin preparation various methods may be used:

- Chemical analyses.
- Determination of biological activity
- Crystallization.
- Studies of solubility
- Studies of the sedimentation constant.
- Spectrophotometric studies.
- Electrophoretic studies.
- Chromatographic studies.
- Iso-electric focusing.
- Immunological studies.

The demands made by the insulin manufacturers on the purity and biological activity of insulin before its final preparation differ widely from place to place. Highly purified insulin

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2. extraction and purification of insulin
3. physical chemical and biological testing of the insulin crystals

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	Depot (Horm)®							0 6 mg protamine 0 15 mg Zn	1	5	18
	Semilente							0 2–0 25 mg Zn	1	5	14
	Lente		A & C					*0 2–0 25 mg Zn*	1 /	8	24
	Monotard®		A & C					0 2–0 25 mg Zn	1 /	6	18
	Depot(Hoechst)®				+			0 4 mg Surfen®	1 /	4	16
	KOMB(Hoechst)							0 28 mg Surfen®	1	3	14
	Globin insulin							2–4 mg globin 0 3 mg Zn	1 /	5	16
								–			
SLOW	ZPI (zinc protamine insulin)							1 25 mg protamine (2 mg Z	3	10	48
	Ultralente							0 2–0 25 mg Zn	3	10	72
	Long-insulin		A & C					0 12 mg Surfen®	3	8	26
BIPHASIC	Initard							*0 15–0 3 mg protamine*	/	5	18
	Rapitard									7	24

bioassays (99) Therefore a species specific standard should be established as soon as possible.

3. Differences between insulin preparations

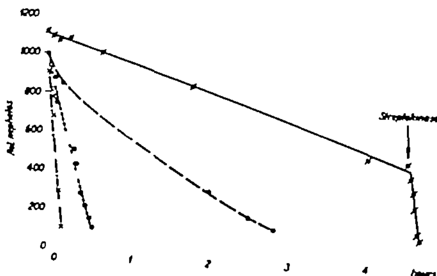
At least 38 different insulin preparations are available on the world market. The most important properties by which they differ from each other are

Duration of effect
Ability to obtain stable mixtures
3 Strength
4 Physical state
5 Species specificity
6 pH
7 Degree of purity

1 Duration of effect

With improvement in the purity of commercial insulin preparations the duration of hypoglycaemic action after subcutaneous injection was found to decrease. To spare the patients having to inject insulin 4 times daily insulins of a prolonged action had to be developed apart from the usual short-acting insulins. After many different approaches (50), a turning point was reached in 1936, when protamine insulin was developed (74). Later Surfen insulin (49), globin insulin (139), phenylisocyanate insulin (75) zinc insulins (76) and low-zinc bovine insulin crystalline suspension (146) were added. The long-acting insulins in use to-day are mainly protamine insulin, zinc insulins and Surfen insulins. Globin insulin and low zinc bovine insulin

Fig 4
Influence of serum, Zn and streptokinase on the disintegration of NPH-insulin crystals The process of disintegration was followed by nephelometer and expressed by relative nephelos
o——o Cryst from 1 ml Insulin Retard NPH + 1 ml serum dilut + 40 µg Zn++
•- - -• Cryst from 1 ml Insulin Retard NPH + 1 ml serum dilut + 20 µg Zn
o o Cryst from 1 ml Insulin Retard NPH + 1 ml serum dilut + 0.5 ml 0 9 per cent NaCl
X- - -X Cryst from 1 ml Insulin Retard NPH + 1 ml serum dilut + 1000 units streptokinase
(from Poulsen & Brunfeld 1953)



should be a glucagon-free crystalline white powder having a proinsulin content below 0 02 % On gel filtration it should yield only one fraction and there must be no bands other than those representing insulin and desamido insulin on acrylamide gel electrophoresis of 150 μg of the powder (cf Figs 1 and 2)

The pharmacopoeias – British Danish as well as American – make demands mainly on biological activity The biological activity is tested by measuring the insulin-induced fall of blood glucose in mice or rabbits or by determining the dose of insulin which induces hypoglycaemic convulsions in mice and comparing the effect with that of the fourth international porcine-bovine insulin standard or a substandard derived from it This met hod is expensive and inaccurate There is evidence that there are differences in the bio logical activity of different species insulins as determined by the mouse and the rabbit in

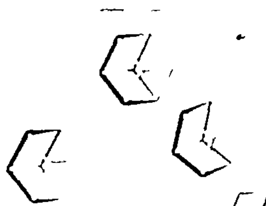


Fig 3 a
Photomicrograph of porcine insulin crystals

Fig 3 b
Photomicrograph of protamine insulin crystals (NPH Leo Reta d) The length of each crystal is 6-7 μ

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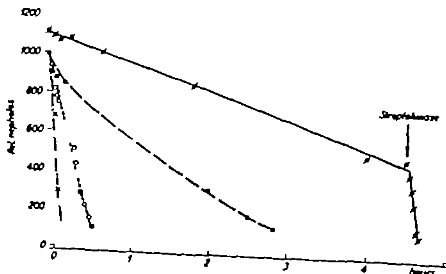
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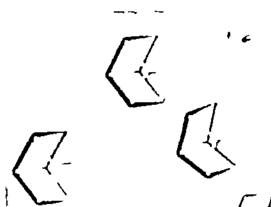


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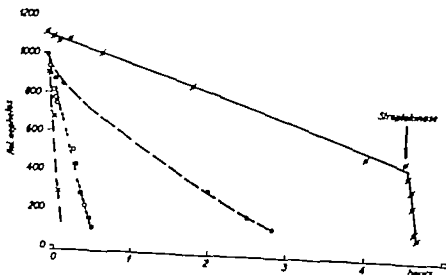
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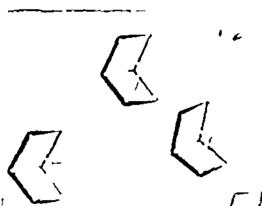


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9 *Strength*

Apart from the difference in duration of action the insulin preparations differ also in biological strength which may vary from 10 to 100 International units per ml. In many patients this unnecessarily wide range has resulted in dosage errors. Fortunately efforts are now being made at producing insulins of only one potency Opinions are deviding into two camps: U.S.A which maintains that the insulin preparations should contain 100 i.u./ml, and Europe, which cannot agree entirely but supports largely a content of 40 i.u./ml. The basis for not accepting 100 I. /ml is primarily the experience that the insulin requirement of diabetics has been decreasing gradually a the purity of the insulin preparations has increased (Table 2). In addition it is difficult to measure off the dose of insulin for young child eceiving e.g. 3 i.u in the evening, if the insulin preparation has strength of 100 i.u./ml Indeed, in the U.S A it has proved necessary to produce diluted preparations for such cases.

> *Physical state*

For therapeutic use the insulin is either dissolved or suspended in a sterile solvent. All short-acting insulins are clear colourless solutions, but all clear colourless solutions of insulin are not short-acting (.g. Surfen insulin, globin insulin and protamine insulin from Hormon Chemie) The use of insulins in solution obviates the potential source of error which arises when drawing insulin suspensions into the syringe However soluble insulin preparations with prolonged action have the drawback that they can be stored only at an acid pH. When they are injected subcutaneously, the solvent will be neutralized and the insulin compound precipitates as amorphous particles of different sizes. Insulin suspensions consist either of amorphous or/and crystalline particles of different sizes or of mixtures of dissolved insulin and a suspension of insulin crystals (Table 1). It must be assumed that a suspension of uniform small crystals is the best presupposition of a reproducible absorption of insulin preparations with prolonged action.

Table 2
Frequency of high and low daily dose of insulin in normalweight diabetics over 18 years of age treated with insulin at different times

		units/day	
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1942-52	500	5.8 %	53.4 %
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Species specificity

Previously the insulin preparations were, as already mentioned predominantly mixtures of bovine and porcine insulin During recent years, however there has been a tendency to keep porcine and bovine insulin separate (the so-called monospecies preparations) The reason is that allergic side effects can not uncommonly be treated with pure porcine insulin and that it has proved possible to produce porcine insulins having little or no immunogenic properties. This has not so far been possible with bovine insulin.

pH

Insulin preparation are either dissolved at an acid pH or dissolved and suspended at a neutral pH The hydrogen ion concentration of the insulin preparations influences their stability, solubility immunogenicity and presumably also the reaction of the tissue. Moreover, intracutaneous injection of acid solutions gives rise to pain.

Formerly when insulin might be contaminated with minimal quantities of enzyme insulin was more stable at an acid than at a neutral pH To-day with the more purified insulins it is the other way Upon storage at 25°C for 500 days, the fall in biological activity is 20 % (pH 3) and 10 % (pH 7 4) respectively The

suspensions are used only on a small scale and phenyl isocyanate insulin is no longer on the Scandinavian market.

Protamine insulin which were developed in Nordisk Insulin Laboratorium (74) are salt-like compounds between the acid poly peptide insulin and the alkaline polypeptide protamine which as shown by Krayenbühl and Rosenberg can be crystallized (97) These insulins are now used almost exclusively in the form of a neutral suspension of protamine insulin crystals the so-called NPH insulin (Fig. 3) (*N*eutral *P*rotamine *H*agedorn) or isophane insulin The prolonged effect of NPH insulin is presumably due to the binding between the insulin and the protamine component being broken down by the fibrinolytic enzymes of the tissue whereupon the insulin component is dissolved (Fig. 4) (29) Protamine is made of fish sperm. It consists primarily of the amino acid arginine and is stated to be devoid of immunogenic properties (70 90) provided that the product is sufficiently purified If the zinc content of protamine insulin is raised from 0 02 mg/100 units to 0 2 mg/100 units and the amount of protamine is increased the result is a zinc protamine insulin in which the prolonged effect of protamine insulin is very considerably enhanced This preparation is available partly as a suspension of crystals partly as a suspension of amorphous particles

The zinc insulins were developed by Novo Ltd after it had been observed that insulin and zinc in an acetate buffer form a complex compound which is slightly soluble in the tissue fluid (76) By varying the physical state of the zinc insulins a varying duration of effect is obtained. Suspensions of amorphous zinc insulin particles (Semilente) have a shorter duration of effect than suspensions of large zinc insulin crystals which have a very long duration of effect (Ultralente)

The effect of a mixture of amorphous and crystalline particles is of intermediate duration (Lente).

By utilizing the difference in the solubility of porcine and bovine insulin Schlichtkrull of Novo has developed a preparation which contains dissolved porcine insulin in a suspension of bovine insulin crystals (146)

Surfen® insulins were developed by Hoechst by forming a complex compound between the synthetic bis (4-aminochinaldine-6)-N N-urea hydrochloride (Surfen®) and insulin (49) The prolonged action arises because amorphous insulin-Surfen particles are slightly soluble in the tissue fluid (Depot Insulin®) Addition of short-acting insulin to this preparation reduces the degree of prolonged action (Komb Insulin®) Only one case of Surfen® allergy is on record (100)

Globin insulins were developed in the Wellcome Laboratories (139) Instead of protamine or Surfen® bovine or human globin was coupled to insulin. The prolongation of the effect is due to the slight solubility of globin insulin in the tissue fluid

Table 1 (see p 200) lists the different durations of action of various insulin preparations. The differences must be considered approximate as the prolonged action of the same insulin preparation depends upon the magnitude of the dose (64) and the absorption from the subcutaneous tissue (25) As a rule a distinction is made between 3 degrees of duration

- short-acting insulin
- intermediate-acting insulin and
- long-acting insulin

By mixing insulin of different types the duration of action may be further modified As a rule however, the rapid initial effect of short-acting insulin will be lost on mixing short-acting with intermediate or long-acting insulin preparations (owing to a surplus of zinc or protamine) Stable mixtures of short-acting insulin and intermediate or long-acting insulin can only be prepared by mixing short-acting insulin with NPH and/or

crystalline ZPI or by mixing short-acting insulin with low zinc bovine insulin crystal suspensions.

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True Binder (25) found a significant difference between the disappearance of radioactive insulin from the subcutaneous tissue when comparing bovine insulin in acid solution with porcine insulin in neutral solution However this difference may have been due to the lesser solubility of the bovine insulin Our investigations indicate that the hypoglycaemic effect of insulin sets in at the same rate whether porcine insulin is administered dissolved at acid or at neutral pH (Fig 5) Accordingly Galloway and Root (63) found no difference in the serum insulin concentration following subcutaneous injection of acid and of neutral insulin. There is some evidence for a role of the pH in the immunogenicity of the insulin preparations cf Fig 22

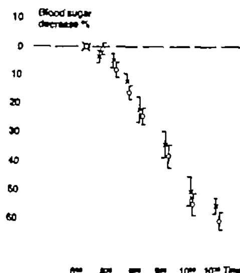


Fig 5
Hypoglycaemic activity of porcine insulin in 9 diabetic patients previously not treated with insulin (cross-over test)
x = *crystalline porcine insulin in acid solution*
o = *crystalline porcine insulin in neutral solution mean ± SEM*

Degree of purity

Insulin preparations differ in their degree of purity The insulin itself and also the retarding substances such as protamine Surfen® and globin may differ in purity Little has been done in the way of investigating the purity of the added substances whereas that of insulin has been studied extensively (147) At present the most common methods for testing the degree of purity of insulin are gel filtration and acrylamide gel electrophoresis (cf p 201) Other methods used are isoelectric focusing and radioimmunological determination of proinsulin and proinsulin-like compounds. If an insulin preparation gives only one peak in gel chromatography on Sephadex G 50 in 1 molar acetic acid it is called monotop insulin, single peak insulin, or chromatographisch reines insulin If analysis of 150 μg crystalline insulin gives rise to only one visible band after staining with Coomassie brilliant blue in acrylamide gel electrophoresis it is called monocomponent or single-component insulin Since the degree of purity of insulins may affect its immunogenicity and tendency to induce subcutaneous lipoatrophy the purity should be very high It is desirable therefore that all marketed insulin preparations should be labelled not only with timing strength physical state species and pH but also with extended purity criteria, including proinsulin content

Special insulin preparations

For the treatment of insulin allergy (cf p 236) various special insulins are available The aim is to reduce the reactivity against

insulin antibodies by changing the molecular structure of the insulin. Dealanine insulin is a porcine insulin in which the terminal amino acid alanine in the B chain has been removed (98). This increases the similarity to human insulin. Sulphated insulin is a preparation containing porcine or bovine insulin treated with sulphuric acid (124 164). However it is doubtful whether the use of these preparations affords any advantage above highly purified porcine insulin.

4. Administration of insulin

Short-acting insulins may be administered intravenously, intramuscularly or subcutaneously regardless of the pH of the solvent. Long or intermediate acting insulins, regardless of whether they are dissolved or suspended in the vial, should not be administered intravenously because of a risk of emboli. Short-acting (clear) insulin preparations may be added to infusion fluids of amino acids, glucose, and electrolytes. On the other hand it is not advisable to add insulin to blood or serum infusion preparations. The reason why insulin should not be added to blood intended for transfusion is that the latter may contain haemolysat which degrades the insulin (28, 36). The reason why insulin should not be added to serum is that the latter may contain enzymes which degrade the insulin. The concentration of insulin in infusion fluids does not affect the stability of the mixture. The amount of insulin to be infused hourly into patients with diabetic ketoacidosis is about 1.2 m-unit per kg/min, which gives a plasma insulin concentration of around 100 μ unit per ml (91 151). It must be mentioned however that marked absorption of insulin to glass and infusion set occurs (32 173). Accordingly it is recommended to add 0.1 % human albumin to the infusion fluids or to anticipate a 25-50 % loss (32). If 50 g glucose is infused in 1 l of water in the course of 4 hours to an adult patient incapable of endogenous insulin production, it is advisable to add 32 i.u. insulin (= 0.8 ml of a 40 i.u. per ml solution) (Fig. 6).

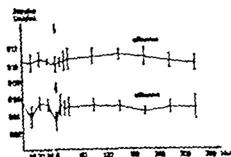


Fig 6

Mean insulinconcentration ± standard deviation of 1100 ml physiologic saline mixed with 110 Units porcine insulin before and during infusion.

Upper curve with 0.1 % human albumin added.

Lower curve without human albumin.

(B Enk and C Eff Unpublished results NSH 1975)

Intravenously injected insulin acts instantaneously. Five min after an intravenous injection of insulin a fall of blood glucose may be recorded in diabetics (Fig. 7). It has not been definitely elucidated how high a plasma insulin concentration is required to obtain a maximum rate of lowering the blood glucose. Presumably it is about 100 μ units per ml (91). Interruption of the insulin infusion rapidly leads to a fall of the plasma insulin concentration and a rapid reduction of the insulin effect upon the plasma glucose concentration (Fig. 7) (151).

After *intramuscular* administration short-acting insulin is absorbed about twice as rapidly as after subcutaneous injection (26). Therefore, intramuscular insulin therapy has come into use in the management of ketoacidosis in cases where continuous intravenous infusion of insulin cannot be established (4).

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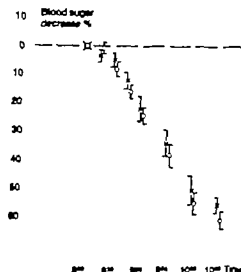


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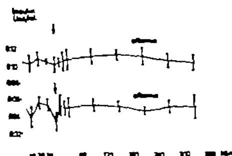


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After *subcutaneous* injection the absorption of short-acting insulin varies considerably. The rate depends upon the region injected (25 26). Besides there are marked individual

explanation is a marked hydrolysis of amido groups (23 63) When kept at 4-5°C all tested insulin preparations have been stable for 30 months (160) Some long-acting insulins are soluble at acid pH (vide supra) However, these insulin compounds will precipitate after subcutaneous injection because of the neutralization of the solvent. The pH of the solvent probably does not influence the rate of absorption from the subcutaneous tissue although insulin dissolved at acid pH has to pass the iso-electric point of insulin after the injection and thereby may be precipitated before being redissolved and absorbed into the blood stream

True Binder (25) found a significant difference between the disappearance of radioactive insulin from the subcutaneous tissue when comparing bovine insulin in acid solution with porcine insulin in neutral solution However this difference may have been due to the lesser solubility of the bovine insulin Our investigations indicate that the hypoglycaemic effect of insulin sets in at the same rate whether porcine insulin is administered dissolved at acid or at neutral pH (Fig 5) Accordingly Galloway and Root (63) found no difference in the serum insulin concentration following subcutaneous injection of acid and of neutral insulin There is some evidence for a role of the pH in the immunogenicity of the insulin preparations cf Fig 22.

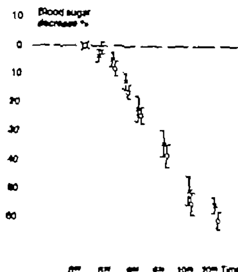


Fig 5
Hypoglycaemic activity of porcine insulin in 9 diabetic patients previously not treated with insulin (cross-over test)
x = *crystalline porcine insulin in acid solution*
o = *crystalline porcine insulin in neutral solution mean ± SEM*

Degree of purity

Insulin preparations differ in their degree of purity The insulin itself and also the retarding substances such as protamine Surfen® and globin may differ in purity Little has been done in the way of investigating the purity of the added substances whereas that of insulin has been studied extensively (147) At present the most common methods for testing the degree of purity of insulin are gel filtration and acrylamide gel electrophoresis (cf p 701) Other methods used are isoelectric focusing and radioimmunological determination of proinsulin and proinsulin-like compounds If an insulin preparation gives only one peak in gel chromatography on Sephadex G 50 in 1 molar acetic acid it is called monotop insulin, single peak insulin, or chromatographisch reines Insulin. If analysis of 150 µg crystalline insulin gives rise to only one visible band after staining with Coomassie brilliant blue in acrylamide gel electrophoresis, it is called monocomponent or single-component insulin Since the degree of purity of insulins may affect its immunogenicity and tendency to induce subcutaneous lipoatrophy the purity should be very high It is desirable therefore that all marketed insulin preparations should be labelled not only with timing, strength physical state species and pH but also with extended purity criteria including proinsulin content

Special insulin preparations

For the treatment of insulin allergy (cf p 736) various special insulins are available The aim is to reduce the reactivity against

variation of the insulin concentration in the serum after subcutaneous injection of intermediate-acting insulin. However, these studies too have shown very pronounced individual variations (26, 68, 116). There is little doubt that these variations must contribute to the difficulties in controlling diabetics.

Other routes of administration

Repeated attempts have been made to arrive at a form of insulin that can be absorbed from the intestinal tract. These attempts have until now been unsuccessful, since insulin is destroyed in the gastrointestinal tract by enzymes and bacteria. Galloway and Root

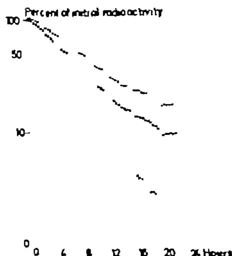


Fig. 8

Disappearance of radioactivity after subcutaneous injection of 8 Units porcine NPH-insulin made from ¹²⁵I-labelled porcine insulin. The preparation was injected in the femoral region. Mean ± standard deviation of 22 measurements in 4 male diabetics. T_{25} = 3 1/2 h, T_{50} = 6 1/2 h, T_{75} = 11 1/2 h. T_{25}, T_{50} and T_{75} express the space of time until 25 %, 50 % respectively 75 % of the injected insulin was absorbed from the subcutaneous tissue.

(H. Dr. [illegible] Petersen, unpublished results NSH 1975)

demonstrated that the plasma insulin concentration can be raised by administering by mouth 8-10 i.u. insulin per kg body weight, together with a surface-active substance such as polyoxyethylene oleyl ether. However, the results were capricious and the intake caused nausea (63).

Elimination of insulin

Insulin is eliminated from the organism by a breakdown of the disulphide bridges. The A and B chains formed in this process are then broken down by proteases into amino acids (169). The enzyme which catalyses the first step of the process (the splitting of the disulphide bridge) is a so-called "insulinase": glutathione insulin transhydrogenase, which has been demonstrated in all tissues studied, but in the highest concentration in the liver and kidneys (169). Thus the hepatic insulin clearance in young adults is stated to be 400 ml per minute, the renal clearance 190 ml per minute, and the insulin clearance from peripheral tissue about 130 ml per minute (151). These findings agree with a total metabolic clearance rate (the plasma volume which is completely and irreversibly cleared of insulin per minute) of 780 ml per minute per 1.73 m² and basal pancreatic insulin production of 24 I.u./24 hours per 1.73 m² (151). About 0.05 % of the daily insulin production is excreted unchanged in the urine (88), little more in the bile (42). 47 % of the insulin which is secreted from the pancreas will be retained during its first passage through the liver (61). The elimination of insulin is presumably a first-order reaction (180), meaning that the absolute degradation rate for insulin is proportional to the concentration of insulin in the plasma. It has been demonstrated that the insulinase system is subject to metabolic control, the glutathione insulin transhydrogenase activity in the liver decreasing in insulin deficiency (169). However, this is not indicated by experiments infusing insulin into diabetics before and 8 days after the institution of insulin therapy (Fig. 7).

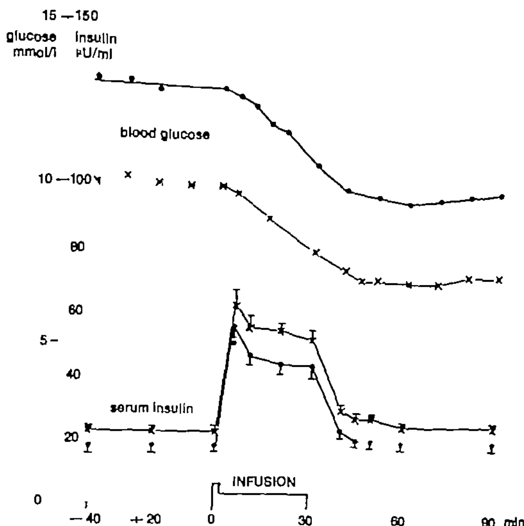


Fig 7
Blood glucose and serum insulin before during and after infusion of 0.8 mU-insulin/kg/min in 11 acetonuric diabetics before and 8 days after insulin treatment The mean of the blood glucose concentration is given and mean ± SEM of serum insulin.

variations Measuring the disappearance of radioactive insulin from the subcutaneous tissue Binder (26) found the residual activity at the injection site 4 hours after the injection of 12 i u insulin Novo into the femoral region to range from 6 to 67 % Moreover a marked day-to-day variation was found in the same person. The variation coefficient for the period elapsing before 50 % of short-acting insulin had been absorbed was about 10 % The variation in the absorption of intermediate-acting insulins such as Lente and Rapitard was – still in the same patient – considerably greater the residual activity 24 hours after subcutaneous injection of radioactive Lente insulin being 40 % ± 14 % (m ± s d) (26) of radioactive porcine NPH-insulin 9 5 % ± 8 % (m ± s d.) (Fig. 8) Not uncommonly there will be periods during which the process of absorption following intermediate-acting insulin appears to have come to a complete standstill (26) On the other hand the absorption seems to be independent of physical activity environmental temperature, and the severity of the diabetes (26) There have been only a few studies on the

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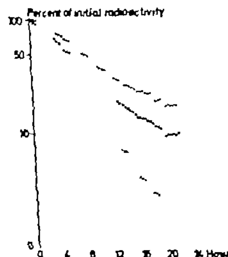


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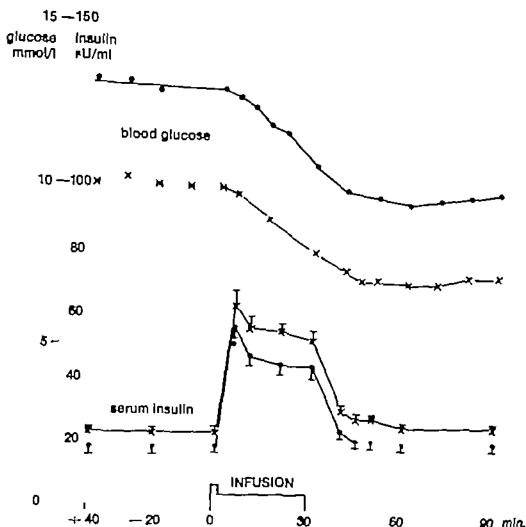


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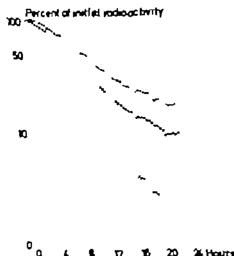


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Intravenously injected or infused insulin disappears rapidly from the blood stream having a half life of about 5-10 min (180) independent of whether the patient has diabetes but longer in the elderly (180) However the half-life of insulin in the plasma is not of major interest as insulin acts chiefly outside the blood stream The distribution space of insulin is 157 ml per kg, corresponding to the distribution volume of inulin and thereby to the extracellular water compartment (169) Thus the distribution space is considerably greater than the vascular plasma volume of insulin 45 ml per kg (169) However the rate at which and the degree to which the insulin molecules from the plasma pass into the interstitial water compartment of the muscles and adipose tissue and back does not depend merely upon the rate at which the insulin concentration in the plasma rises or falls but above all upon to what level the plasma insulin concentration rises and how long the change in concentrations lasts (169)

Insulin is not bound to plasma proteins (22) (24) unless insulin binding antibodies are present

Insulin treatment

1 Object of the treatment

Diabetes mellitus is characterized partly by metabolic disorders leading to the classical symptoms and signs, partly by tendency to develop neuropathy microangiopathy and arteriosclerosis manifesting themselves in various organic lesions.

The object of treatment is to attain well-being and to make the patient s organism function normally physically and mentally – not only at the moment, but also at longer sight. This seems to be best secured by aiming at re-establishing normal physiological conditions (*vide infra*)

Reversible metabolic and functional disorders

Normally the concentration of blood glucose shows only minor changes owing to a normal interaction between insulin, glucagon, and the liver The diabetic organism is characterized by unsatisfactory function of this interaction. Fig 9 illustrates the capillary blood glucose concentration after breakfast in normals and in insulin-treated diabetics. It is apparent that despite an adequate supply of insulin during the hours of the night, and despite the injection of insulin 30 min before breakfast diabetics exhibit a far greater increase in blood glucose than normals. The reason is partly that the mass of their islet tissue is too small (66 176) partly that what remains of the islet tissue cannot release insulin soon enough and not in sufficient quantities (88 135). This insufficient supply of insulin entails not only a constant or transient hyperglycaemia, with its consequences (Tables 3 and 4) but a large number of other abnormalities (Table 5) which may be normalized if it proves possible to obtain a normal supply of insulin to the various organs for a long enough period

Irreversible late diabetic changes

The cause of persistent neuropathy, microangiopathy and arteriosclerosis has not been definitely elucidated, but various factors indicate that the metabolic abnormalities caused by the insulin deficiency play a decisive role. For a detailed discussion cf Field (62).

It is also unknown which of the abnormal metabolic and physiological factors due to the insulin deficiency should be attributed with most pathogenetic importance in the development of late diabetic manifestations. Presumably an interplay of the above-mentioned factors (Tables 3 and 4) is responsible

No direct proof can be adduced that late diabetic manifestations can be prevented by diabetes control, int. al. with blood glucose levels within physiological limits maintained during life However we have today cumulative evidence showing a causal relationship between the abnormal metabolism due to insu-

Table 3
Intermittently abnormal concentrations of plasma metabolites and hormones in uncomplicated unstable insulin-treated diabetics

glucose (123) lactate (3)	haemoglobin (46 47) Hb A_{1c} (134 165) erythrocytic 2 3 DPG (46, 47)
acetoacetate (142) β-hydroxy butyrate (159) glycerol (3) free fatty acids (127) triglycerides (127 159)	proconvertin (168) insulin antibodies (39)
amino acids (59) albumin (144) H (83 46) pCO_2 (83)	insulin (123) glucagon (15) growth hormone (78) catecholamines (39) cortisol (16)

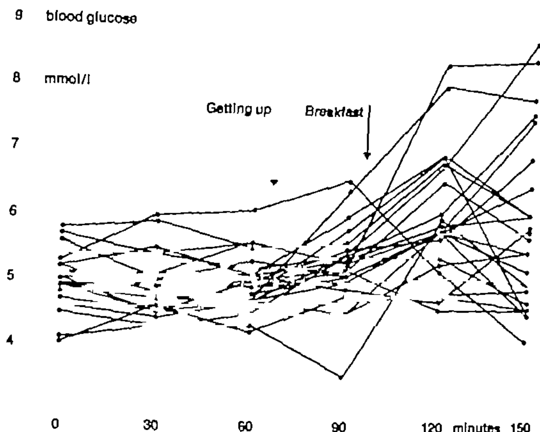


Fig 9 a
Variations of capillary blood glucose concentration after breakfast in 25 non-diabetics Between 0-60 min the subjects were lying in bed The time of getting up and of breakfast are indicated by arrows

lin deficiency and late diabetic manifestations (27 51 84 104 152) Therefore the endeavour to obtain normal physiological metabolic conditions must be considered even in patients who are feeling well despite a high blood sugar To attain this goal in treating diabetes it must be attempted to obtain the best biochemical control that can be practised by easily accessible methods with due regard to the patient s safety and abilities

Table 4
Processes induced by high glucose concentration

sorbitol accumulation (175)
activation of glucuronic
acid cycle (19) (174 156)
reduced leucocyte function (17)

Insulin supply an integral part of diabetes treatment

A complete metabolic normalization is obtained by providing the various tissues of the diabetic organism with an optimal quantity of insulin at the right time This has been demonstrated by using a so-called artificial endocrine pancreas (5) and by transplantation of pancreas to patients with long-lasting juvenile diabetes mellitus (107) However these methods are not yet practicable as long-term treatment of diabetes and we must still resort to the methods used so far to obtain metabolic control

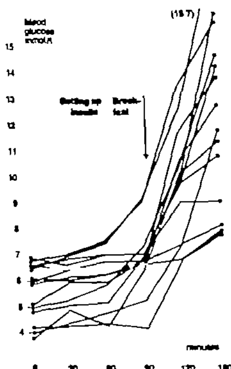


Fig 9 b
Variations of capillary blood glucose concentration after breakfast in 14 insulin-treated diabetics. Between 0-60 min the subjects were lying in bed. Th time of getting p nd of breakfast are indicated by arrows

Metabolic control in diabetes can be influenced by

- ( ) endogenous secretion of insulin,
- (b) insulin sensitivity
- (c) food intake and exercise,
- (d) exogenous administration of insulin.

A systematic review of the various factors influencing the metabolic control will not be given here but it must be emphasized that insulin treatment should always be considered integrated with all the other factors of significance in treating diabetes.

Table 5
I termittently disturbed f nctions in uncomplicated unstable diabetics

microvascular permeability (133)
glomerular filtration rate (120)
serum viscosity (114)
resting blood flow in the forearm
and subcutaneous tissue (73 130)
heart rate (73 37)

leucocyte phagocytosis (17)
coagulation processes (168)

nerve motor conduction velocity (71 170)
vibration sense (158 163)

Insulin therapy

Treatment with insulin has meant a fantastic advance to young diabetics whose life has been prolonged by 30-35 years. However in view of the aim, which is complete metabolic control, treatment with insulin as practised to-day must be considered an unsatisfactory substitution therapy. It is true that it can satisfy the erage daily insulin requirement but insulin therapy cannot reproduce the delicately balanced insulin secretion in normals. As has been said insulin is given in the wrong quantity at the wrong time and in the wrong site.

I non-diabetics the endogenous insulin secretion is subject to extremely effective regulation primarily by the blood glucose concentration, intestinal hormones, and the autonomic nervous ystem (111). Thereby it is possible, at seconds notice to reduce or arrest the insulin secretion according to the instantaneous requirement of the organism. Combined with the short biological half-life of insulin, about 10 min, and its excretion to the portal vein, these factors secure a very effective control of blood glucose, not only during fasting, but also after meals, during physical activity stress, infection, pregnancy etc.

In diabetics the regulation of endogenous insulin secretion has broken down (cf. Chapter 3). Subcutaneous administration of insulin is

9 blood glucose

8 mmol/l

Getting up Breakfast

7

6

5

4

0 30 60 90 120 minutes 150

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8 — pregnancy, oral contraceptives, menstruation,
9 — factors influencing the elimination of insulin (cirrhosis, uremia),
10 — intercurrent diseases
11 — degree of metabolic decompensation
12 — hormones affecting insulin sensitivity
13 — drugs affecting insulin sensitivity
14 — anxiety

Fig. 11 shows the variation of the 24-hour dose of insulin in normal-weight diabetic children. The 24-hour dose per kg body weight is constant during the growing period (0.8 units/kg). Adult diabetics above 18 years of age have a lower daily insulin requirement than adolescents between 14 and 18 years, absolute as well as per kg body weight. The explanation is presumably in part that among the adults there are more insulin-treated diabetics having more or less preserved endogenous insulin production than among children and in part that insulin-treated children have a higher insulin antibody titre than adults (7).

(2) The greater the endogenous insulin production the less exogenous insulin is required. It has been recognized for many years that quite often the insulin requirement of young diabetics decreases after the diabetes has been diagnosed and treated initially. This period of remission or "honeymoon", lasts from weeks to a couple of years. Its cause does not seem to be an increased insulin sensitivity (Fig. 7) but an increased endogenous insulin production (Fig. 12). Therefore, almost 2/3 of young diabetics with an onset before the age of 30 and a duration of less than 2 years could manage on insulin once daily as compared with only about 1/4 of juvenile diabetics with a duration exceeding 2 years. Cessation of endogenous insulin production is presumably also the explanation of the so-called "Spatversager", i.e. the phenomenon that some patients treated with sulphonylurea cease responding to the tablets after some years' treatment.

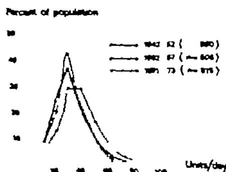


Fig. 10
Daily insulin requirement in diabetic patients during 3 different periods. All the patients were ambulatory and over 18 years of age without fever, pregnancy or uremia and had been treated with insulin for more than one year

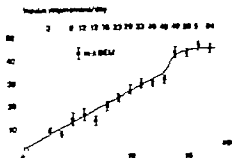


Fig. 11
Mean daily insulin requirement in children of different age. All the children were ambulatory and had been treated with insulin for more than one year
Φm ± SEM n = number of children investigated

(3) The influence of the diet upon the insulin requirement is known to all diabetologists and has been amply substantiated in animal experiments (67). The insulin requirement decreases during fasting.

(4) In juvenile diabetics with a long duration of

merely an attempt to reproduce the fantastic interplay of nature But so far this has not proved possible In non-diabetics the plasma insulin level is high when the blood sugar is high and low when the blood sugar is low whereas in most insulin-treated diabetics the opposite applies i e the plasma insulin level is low when the blood sugar is high and *vice versa* (122)

Indications

Insulin treatment must be instituted when the metabolic disorders caused by insulin deficiency threaten life This is so in ketoacidosis and non-ketotic hyperosmolar coma. Moreover insulin treatment must be instituted when acceptable metabolic function cannot be achieved by increasing the endogenous insulin production increasing the insulin sensitivity and reducing the insulin requirement.

What is taken to be acceptable metabolic function is still a matter of discussion Most investigations show that even very mild degrees of diabetes (reduced glucose tolerance) are attended by a reduction in the quality and length of life (45) Since however there is no causal relationship between the prognosis and the hyperglycaemia especially in elderly people with fairly mild diabetes (167) it is not possible at present to give any general statement as to how far the blood glucose has to be reduced to attain a therapeutic gain At the Steno Memorial Hospital in Copenhagen we have so far accepted a postprandial blood glucose around 11 mmol/l (corresponding to 198 mg/100 ml) somewhat lower in young and somewhat higher in elderly people Besides regard must of course be paid to special factors such as pregnancy infection wounds gangrene etc and to the patient s well being safety and abilities In this connection it must be mentioned that the glucose concentration in the blood is not always a sufficiently good marker of metabolic control since the concentration of ketone as well as of lactate may be considerably elevated despite a blood glucose level below 150 mg/100 ml (3)

Prevalence

Only a relatively small number of diabetics require insulin substitution therapy While about 1.5-2 % of the population in most industrial nations have diabetes (depending upon the diagnostic criteria used) insulin treatment is needed in only 0 3-0 4 % depending on race (35 112) age distribution of the population frequency of heavy manual work standard of living access to insulin and the medical profession s attitude to oral hypoglycaemic agents and demands on the quality of the treatment In Denmark too there are still obese patients on unnecessary insulin treatment and reversely patients who muddle through on oral hypoglycaemic agents instead of utilizing the benefit of insulin treatment.

Daily dose of insulin

Insulin is a 6000 molecular weight polypeptide which is split by the intestinal enzymes. Therefore it has to be administered parenterally So far it has not been possible to produce insulin preparations the absorption of which from the muscles or the subcutaneous tissue could be kept constant through weeks or months. Daily injections are still needed. The daily dose has decreased somewhat through the past 20 years (Fig 10) The explanation is presumably that the antigenicity of the insulin preparations has been decreasing and this results in a lower concentration of insulin-binding antibodies (43) The mean daily dose of insulin for insulin-treated diabetics is lower in Denmark than in Sweden (131) and e g in West Germany (56) The cause is unknown

In each patient many factors are of importance to the optimal daily dose of insulin

1 age
2 – the endogenous insulin secretion
3 – diet
4 – duration of diabetes
5 – antibodies
6 – physical activity
7 – degree of obesity

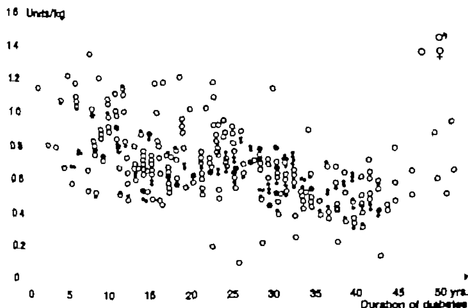


Fig. 13
Daily insulin requirement in 81 adult subjects with diabetes of mor than 40 years duration. All patients had developed diabetes before the age of 30 and had been hospitalized more than 3 times. Each point indicates the daily insulin requirement of one of the 81 patients at the end of one of the admissions

requirement is unknown. The more severe the impairment of renal function the more common are these peculiar undulations (Table 7).

(B) Intercurrent diseases, especially febrile illnesses, increase the insulin requirement [illegible] al because of an increase in the concentration of hormones such as the growth hormone, glucagon, and cortisol which counteract the effect of insulin (65 138) (Fig. 15). During febrile illnesses therefore insulin-treated

Table 6
Daily insulin equirement in 30 juvenile diabetics with diabetic ephropathy before and after erum creatinine as elevated. Mean ± SEM are given.

	Se-creatinine mg/100 ml	insulin (units/day)	insulin (units/kg/day)	caloric intake (kcal)	years between
before	1.03±0 03	44 1±2.4	0.70±0 04	2052 38	
after	5 78±0 90	35.9±1.8	0.59±0.03	1859±45	7 7

mean ± SEM

the disease the insulin requirement decreases (Fig. 13) This is presumably due to the insulin disappearance rate from the plasma (180) as well as the lean-body mass and creatinine clearance decreasing with age (89)

) Insulin antibodies also appear to influence the daily 24-hour dose It has been demonstrated that adult normal-weight patients treated from the onset with highly purified porcine NPH insulin (6 % formed antibodies) had a significantly lower insulin requirement than patients who had been treated initially with pig NPH insulin which was not highly purified (75 % formed antibodies) (43)

That physical activity also in the form of a 7½-minute brisk walk in a well-controlled diabetic has an insulin-saving effect is well-known and amply substantiated (92) The fall of blood glucose is the greater the higher the glucose concentration. In poorly controlled diabetics on the other hand the

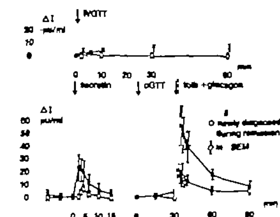


Fig 12
Insulin response during intravenous glucose tolerance test (25 g) after secretin injection (75 units) after oral glucose (50 g) and after injection of tolbutamide (1.0 g) + glucagon (1.0 mg) in 5 brittle diabetics
o = newly diagnosed
x = during remission
y = mean + SEM
ΔI = current insulin concentration less insulin concentration at time o

blood sugar rises during physical activity (52)

(7) Overweight patients usually tolerate very high doses of insulin provided that caloric restriction has not been established The cause of this insulin insensitivity which applies not only to exogenous insulin but also to the patient's own endogenous insulin is unknown The great majority of overweight diabetics are of the maturity-onset type (of the patients of the Steno Memorial Hospital *with onset before the age of 30 only 4 % are* overweight) and in such patients endogenous secretion of insulin is usually high Therefore administration of insulin is usually unnecessary as a near-physiological metabolic state may be obtained by a moderate caloric restriction.

(8) It is well-known that the insulin requirement of pregnant patients rises during the last trimester of pregnancy In insulin-treated diabetics this means that the daily dose of insulin has to be increased gradually by an average of 50-100 % (53) Immediately *post partum* the insulin requirement returns to the pre pregnancy level a phenomenon which indicates the role of the placenta in this connection Many oestrogen-containing oral contraceptives reduce the insulin sensitivity *at least temporarily (154) Therefore the in*sulin dose has to be adjusted in many diabe*tics treated with oral contraceptives Imme*diately before a menstrual period the insulin requirement *may also fluctuate* appreciably Indeed Sandström (145) has described a diabetic who developed ketoacidosis during each menstrual period

(9) (A) Table 6 shows that the insulin requirement in juvenile diabetics decreases when renal function becomes impaired but hardly by more than what corresponds to the decreasing lean body mass and less calorie intake Almost 50 % of patients with impaired renal function have an undulating insulin requirement (Fig 14) which makes it very difficult to control the diabetes The cause of this varying insulin

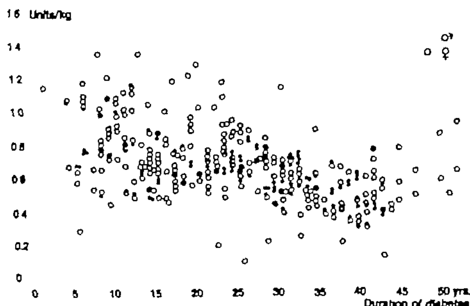


Fig. 13
Daily insulin requirement in 81 adult subjects with diabetes of more than 40 years duration. All patients had developed diabetes before the age of 30 and had been hospitalized more than 3 times. Each point indicates the daily insulin requirement of one of the 81 patients at the end of one of the admissions.

requirement is unknown. The more severe the impairment of renal function the more common are these peculiar undulations (Table 7).

(3) Intercurrent diseases, especially febrile illnesses, increase the insulin requirement (int. al. because of an increase in the concentration of hormones such as the growth hormone, glucagon, and cortisol, which counteract the effect of insulin (65, 138) (Fig. 15). During febrile illnesses therefore insulin-treated

Table 6
Daily insulin requirement in 30 juvenile diabetics with diabetic nephropathy before and after serum creatinine was elevated. Mean ± SEM are given.

	Se-creatinine	insulin		caloric	years
	mg/100 ml	(units/day)	(units/kg/day)	intake (kcal)	between
before	1.03±0.03	44.1±2.4	0.70±0.04	2052±38	
after	5.78±0.90	35.9±1.8	0.59±0.03	1859±45	7.7

mean ± SEM

the disease the insulin requirement decreases (Fig. 13) This is presumably due to the insulin disappearance rate from the plasma (180) as well as the lean-body mass and creatinine clearance decreasing with age (89)

Insulin antibodies also appear to influence the daily 24-hour dose It has been demonstrated that *adult normal* weight patients treated from the onset with highly purified porcine NPH insulin (6 % *formed antibodies*) had a significantly lower insulin requirement than patients who had been treated *initially* with pig NPH insulin which was not highly purified (75 % formed antibodies) (43)

That physical activity also in the form of a 7½-minute brisk walk in a well-controlled diabetic has an insulin-saving effect is well-known and amply substantiated (92) The fall of blood glucose is the greater the higher the glucose concentration. In poorly controlled diabetics on the other hand the blood sugar rises during physical activity (52)

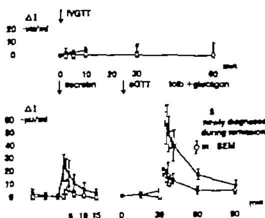


Fig 12
Insulin response during intravenous glucose tolerance test (25 g) after secretin injection (75 units) after oral glucose (50 g) and after injection of tolbutamide (1.0 g) + glucagon (1.0 mg) in 5 brittle diabetics
o = newly diagnosed
x = during remission
y = mean + SEM
ΔI = current insulin concentration less insulin concentration at time o

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had been diagnosed before the age of 30 73 % received insulin twice daily. Only one patient was treated with insulin thrice daily (Table 9). Out of insulin-requiring diabetics whose disease had been diagnosed after the age of 30, 54 % managed on insulin once daily. 96 % of the patients used NPH Insulin. 35 % of the patients, especially among those who received insulin twice daily used a combination of short-acting and intermediate-acting insulin in the morning. The majority of these patients themselves prepared a combination of NPH and short-acting insulin in neutral solution. Only a few (6 %) used commercially available biphasic insulins, mostly Initard®. Out of the 90 juvenile diabetics who received insulin once daily 35 had had their diabetes for only 1 2 years and 15 for more than 5 years. If insulin is injected twice daily two-thirds of the daily dose is usually given in the morning and one-third in the evening. All insulin injections should be given 30 minutes before meal.

During recent years French authors in particular have strongly recommended giving short-acting insulin thrice daily supplemented by a long-acting insulin in the evening (94)

Table 9
Frequency of daily insulin injections in 319 diabetics treated with insulin for more than one year. The diagnosis of diabetes was in all cases made before the age of 30

Frequency of daily injections	%	% using biphasic insulin
once	26.7	5 %
twice	73.0	46 %
thrice	0.3	–

= 319

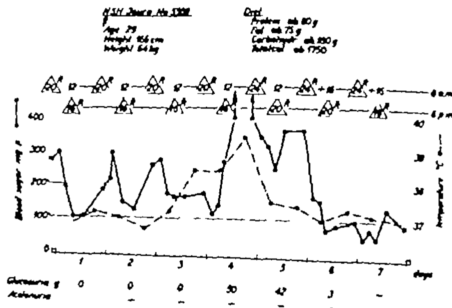


Fig 15
Influence of fever on the blood glucose regulation in a diabetic patient Δ ± 12 = NPH-insulin + 12 units of short-acting insulin*

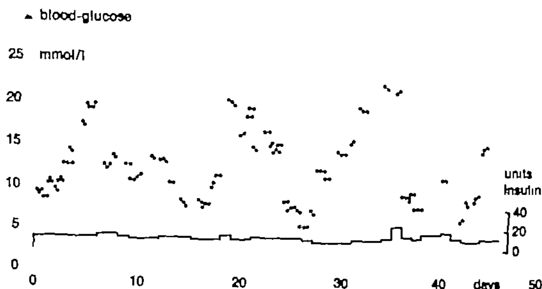


Fig 14
Blood sugar undulations in a 60-year-old diabetic patient with diabetic nephropathy and a serum creatinine of 7.0 mg % Diabetes duration 21 years The insulin dose given once daily was fairly constant No infusions were given The patient was in bed and food intake was fairly constant

diabetics should receive about 25 % more insulin for each degree the temperature exceeds 37 5°C If this is not observed keto-acidosis is apt to occur Infectious diseases are among the most common triggers of keto-acidosis today In severe metabolic decompensation as e g in ketoacidosis the daily insulin requirement is also increased (*vide infra*)

Table 8 lists some of the hormones and drugs which affect insulin sensitivity

Table 7
Frequency of blood sugar undulations in patients with diabetic nephropathy

se-creatinine mg/100 ml	no	number of patients with blood glucose undulation (%)
1.3–2.0	47	17 (35)
2.1 5 0	25	14 (55)
>5 0	11	7 (70)

How many injections daily?

Insulin is injected once to several times daily Whereas many patients who develop diabetes after the age of 30 do very well on one daily dose it has been substantiated that in juvenile diabetics a better regulation of the blood sugar is generally obtained by administering insulin twice or thrice daily (94 181 182) Out of 319 diabetics who had required insulin for more than one year and whose disease

Table 8
Hormones and drugs influencing the requirement of insulin

increasing requirement	decreasing requirement
cortisol	tetracycline (80)
prednisone	salicylates
glucagon	alcohol
growth hormone	biguanides
catecholamine	
thyroxine	
oral contraception	
diuretics	

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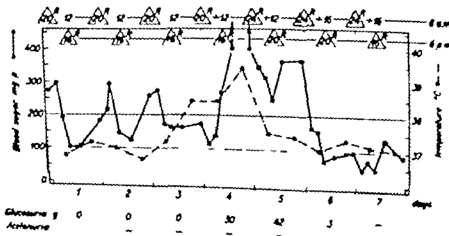


Fig. 15
Influence of fever on the blood glucose regulation in a diabetic patient. A^R ± 12 = NPH-insulin + 12 units of short-acting insulin.

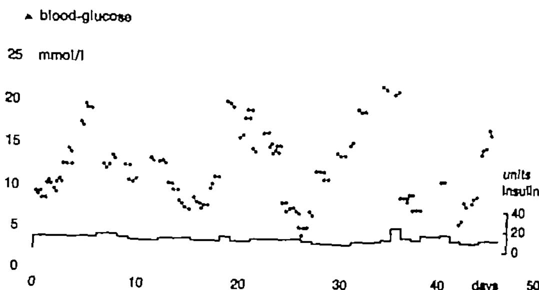


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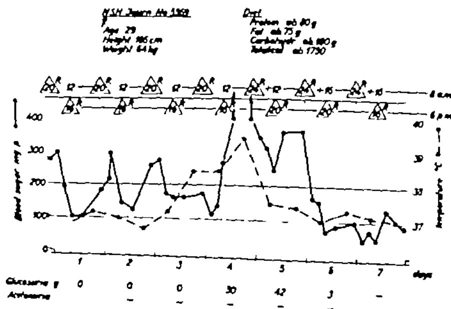


Fig. 15
Influence of fever on the blood glucose regulation in a diabetic patient. Δ^R ± 12 = NPH-insulin + 12 units of short-acting insulin.

However any orthodoxy in this field must be considered unbiological. As already mentioned insulin therapy is an integral part of the treatment of diabetes and regard must be paid both to endogenous insulin production insulin sensitivity and the diet. Especially in patients with maturity-onset diabetes both the endogenous insulin production (e.g. during treatment with sulphonylurea) and insulin sensitivity may be appreciably affected (e.g. by diet and exercise). Therefore a rigid insulin regime must be considered inappropriate. On the other hand there is no doubt that many diabetics with onset before the age of 30 whose endogenous insulin production ceases entirely in the course of a few years could obtain better control of their diabetes by 3 injections daily. Fig. 16 a sets out 24-hour curves of capillary blood glucose in 4 patients who had required insulin for many years. After the usual insulin treatment had been withdrawn short-acting insulin was administered 3 times daily for 48 hours in order to eliminate the effect of intermediate-acting insulin from the subcutaneous depots. Thereafter intravenous insulin infusion was started by the aid of an electrical pump. The infusion was continued at a constant rate for 72 hours. The patient continued on his usual diet i.e. a warm meal at noon and the bread ration distributed as 30 % at 8 a.m. 10 % at 2 p.m. 40 % at 6 p.m. and 20 % at 10 p.m. 100 g fruit was served at 10 a.m. and at noon. All the patients were ambulatory and followed an exercise programme on an ergometer bicycle

½ hour from 10.30 to 11 a.m.
1 hour from 3 to 4 p.m.
½ hour from 6.30 to 7 p.m.

Each curve on the figure indicates the mean blood glucose concentration from 3 consecutive days and nights for each patient. The uniform pattern apparent from the course of the curves shows that a constant insulin supply to the tissues in the course of the 24 hours is not sufficient to keep the blood glucose within physiological limits. This can be attained only when the infusion rate is varied (Fig. 16 b). Fig. 16 b shows how the blood glucose concentration may be controlled by a variable infusion based upon the above experiment. If the insulin infusion pattern de-

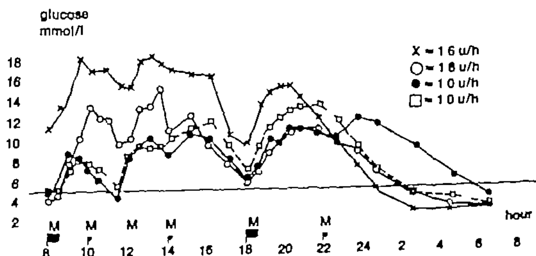


Fig. 16 a
Capillary blood glucose in 4 brittle diabetics during 3 days of continuous insulin infusion. The infusion was given at a constant rate (1.0, 1.0, 1.6, 1.8 units/hour). Each curve represents the mean of 3 days infusion in one individual

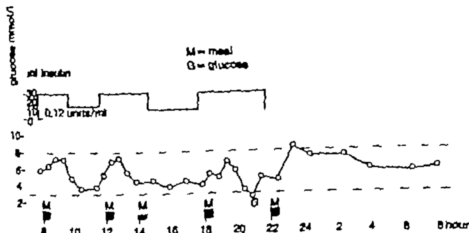


Fig. 16 b
Capillary blood glucose in a brittle diabetic during continuous insulin infusion. The infusion is given at varying rate

scribed above is to be reproduced in subcutaneous insulin therapy. It is presumably best to administer long-acting insulin in the evening, supplemented by 3 injections of short-acting insulin before the chief meals.

Which insulin to choose?

Among the numerous insulin preparations on the market, one should select a palette of insulins applicable in all clinical situations. In the choice of such a pallette the following regards have to be considered:

(1) The palette must contain a short-, an intermediate- and a long-acting insulin.
(2) The insulins must be dissolved or suspended in neutral buffer.
(3) The variously timed insulins must be mutually miscible without the individual components losing their specific individual spectrum of action.
(4) The insulins should have as low antigenicity as possible.

At the Steno Memorial Hospital we generally use Insulin Leo Neutral®, Insulin Retard (NPH) and crystalline zinc protamine insulin, a palette which fulfills all the above-mentioned conditions.

Institution of treatment

In most cases the patient should be in a specialized hospital unit when insulin therapy is to be instituted. This is partly to secure adequate observation and partly to make the patient cognisant of and familiar with the problems connected with insulin treatment.

Only when the abnormal metabolic states due to insulin deficiency are life-threatening, i.e. in ketoacidosis and non-ketotic hyperosmolar coma, does insulin therapy have to be instituted immediately and effectively. In all other cases there is time to prepare the patient by talks and to proceed more gradually.

Insulin treatment in ketoacidosis and in hyperosmolar non ketotic coma

The optimal form of treatment in ketoacidosis and in hyperosmolar coma is continuous intravenous infusion of insulin (91, 137). This is the only way of instantaneously raising and

However any orthodoxy in this field must be considered unbiological As already mentioned insulin therapy is an integral part of the treatment of diabetes and regard must be paid both to endogenous insulin production insulin sensitivity and the diet Especially in patients with maturity-onset diabetes both the endogenous insulin production (e g during treatment with sulphonylurea) and insulin sensitivity may be appreciably affected (e g. by diet and exercise) Therefore a rigid insulin regime must be considered inappropriate On the other hand there is no doubt that many diabetics with onset before the age of 30 whose endogenous insulin production ceases entirely in the course of a few years could obtain better control of their diabetes by 3 injections daily Fig. 16 a sets out 24-hour curves of capillary blood glucose in 4 patients who had required insulin for many years After the usual insulin treatment had been withdrawn short-acting insulin was administered 3 times daily for 48 hours in order to eliminate the effect of intermediate-acting insulin from the subcutaneous depots Thereafter intravenous insulin infusion was started by the aid of an electrical pump The infusion was continued at a constant rate for 72 hours The patient continued on his usual diet i e a warm meal at noon and the bread ration distributed as 30 % at 8 a m. 10 % at 2 p m 40 % at 6 p m and 20 % at 10 p m 100 g fruit was served at 10 a.m. and at noon All the patients were ambulatory and followed an exercise programme on an ergometer bicycle

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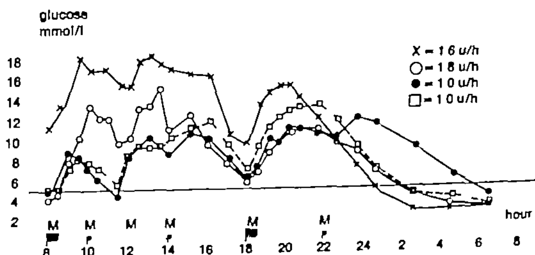


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or else they forget that insulin should be taken half an hour before a meal. If the insulin dose is to be altered, this must be done gradually i.e. it may be altered 10 % to 15 % up or down, and then 3 days should elapse before further changes are made, unless acute situations require the doctor's help. Any changes should be made on the basis of the patient's condition (possible hypoglycaemia) body weight, postprandial blood sugar and urinary excretion of glucose. If the postprandial blood sugar is too high, short-acting insulin has to be combined with the intermediate- or long-acting insulin.

Insulin-treated diabetics should be seen quite often (every month to every 3 months), because many factors may influence the metabolic balance and because the patients may be feeling well for a long time although the biochemical state is chaotic.

When is the follow-up examination satisfactory? In patients with maturity-onset diabetes we try to obtain and maintain:

- freedom from diabetic symptoms,
- freedom from hypoglycaemia,
- working capacity
- postprandial blood glucose concentration <11 mmol/l,
- freedom from glycosuria,
- *freedom from ketonuria*,
- freedom from hyperlipoproteinaemia,
- normal body weight,
- acceptable blood pressure

In patients whose diabetes has set in before the age of 30 or other brittle diabetics, the above-mentioned demands concerning the postprandial blood sugar and glycosuria can generally not be fulfilled. In such patients we aim at postprandial blood glucose concentration below 15 mmol/l and a urinary excretion of glucose below 15 % of the 24-hour carbohydrate intake. In the case of children we add the demand of a satisfactory longitudinal growth and weight increase. Even though these demands are not

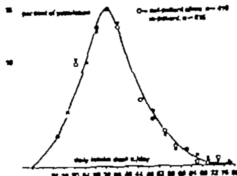


Fig 17
Distribution of daily insulin requirement in 615 diabetic in-patients and 418 out-patients

fulfilled, the insulin dose need not always be altered. Slight changes in the distribution of carbohydrate intake over the 24 hours and/or a change in physical activity is often sufficient. Otherwise the patients may easily be overdosed. At the Steno Memorial Hospital overdosage of insulin is one of the most common causes of a poor diabetes control in newly referred patients.

Discontinuation of insulin therapy

As already mentioned, many factors influence insulin requirements (cf. p. 215). These factors are not invariable in the same individual. Therefore it may happen that a previously necessary insulin therapy can be discontinued. This is not infrequently possible in maturity-onset diabetics started on insulin therapy during a period of intercurrent disease or before a dietetic regime has given a result. The same applies to patients with pregnancy diabetes and young diabetics who go into remission (cf. p. 215). Resumption of the insulin therapy at a later date usually involves no risk of anaphylaxis to-day when most insulins are of a high degree of purity. On the other hand, the development of insulin resistance (cf. p. 234) after intermittent insulin therapy is still a problem in many countries (56). It must, therefore be recommenced *to use insulins of an antigenicity as low as possible*.

reducing the plasma insulin concentration (Fig. 7) Owing to the dehydration and circulatory failure in patients with ketoacidosis and with hyperosmolar coma subcutaneous injection of insulin is often insufficient as the absorption of insulin from the subcutaneous tissue is uncontrollable To be on the safe side very high doses of insulin have been given subcutaneously (up to 5000 i u /24 hours) This has entailed a risk of life threatening hypopotassaemia hyperlactataemia and hypoglycaemia If the insulin therapy is given instead as intravenous injection hourly to every 4th hour there will be established an intermittent insulin deficiency state due to the very short biological half-life of insulin (cf p 209) These disadvantages may be avoided by continuous intravenous infusion of insulin 100 i u of short-acting insulin are dissolved in 1 litre of a physiological sodium chloride solution containing 0 1 % human albumin *The albumin is added to avoid absorption of insulin to the infusion system* If albumin cannot be procured the dose of insulin should be increased by about 25 % in order to compensate the adsorption of insulin to the infusion set (32 173) About 150 ml an hour is infused during the first 2 hours corresponding to 10-15 units/hour thereafter 50-100 ml/hour or 1 mU kg/min This form of treatment secures a constant and effective serum insulin level of about 80-100 μU/ml which lowers the blood glucose by 4-5 mmol/hour (91 151) If continuous intravenous infusion cannot be established intramuscular injection of insulin every hour 10 i u the first 2 times and thereafter 2 5 i u /hour is the next-best method (4) From the 2nd to the 3rd day of the treatment short-acting insulin should be injected subcutaneously 4 times daily for a few days thereafter intermediate-acting insulin. Concerning fluid and electrolyte treatment consult reviews (81)

Institution of insulin treatment in diabetics without ketoacidosis

If the patients have glycosuria and ketonuria intensive observation is indicated either at home or in hospital If the general condition is poor the patient young febrile normal-weight or pregnant insulin treatment is always needed In obese patients in good general condition dietetic treatment may be tried first Insulin is administered subcutaneously as short-acting or intermediate-acting insulin in a dosage of about 0 2 to 0 5 i u./kg several times daily depending upon the general condition

If the patient does not have ketonuria it is justified before instituting insulin therapy to try other possibilities i e improving the endogenous insulin secretion increasing the insulin sensitivity and/or decreasing the insulin requirement If acceptable metabolic control is not obtained (cf p 223) insulin therapy has to be instituted by an intermediate or long-acting insulin in a dose of about 0.2 to 0 3 i u /kg once daily The dose is gradually increased every 3rd to 4th day If acceptable *metabolic control is not obtained on 35 to 40* i u in the morning insulin has to be given also in the evening.

Continued insulin treatment

Most hospital units discharge their patients on too high a dose of insulin This is presumably because the patients are more active physically after returning home from the hospital and because complicating diseases which may have been responsible for a decreased insulin sensitivity are improving. It should be endeavoured therefore to secure as much physical activity during the stay in hospital as permitted by the general condition At the Steno Memorial Hospital this is possible Therefore the daily insulin dose for *the out-patients is not lower than for the* in-patients (Fig. 17) At all events the insulin treatment after discharge from hospital has to be adjusted by an out-patient follow-up In the case of beginners in insulin therapy the injected areas must be checked on this occasion and the injection technique discussed anew Not uncommonly the patients develop bad habits after their discharge from hospital For instance they may *inject the insulin intracutaneously for fear of going too deep*

or else they forget that insulin should be taken half an hour before a meal. If the insulin dose is to be altered this must be done gradually i.e. it may be altered 10 % to 15 % up or down, and then 3 days should elapse before further changes are made, unless acute situations require the doctor's help. Any changes should be made on the basis of the patient's condition (possible hypoglycaemia) body weight, postprandial blood sugar and urinary excretion of glucose. If the postprandial blood sugar is too high, short-acting insulin has to be combined with the intermediate- or long-acting insulin.

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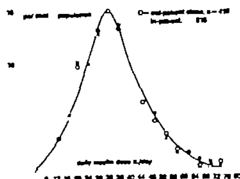


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reducing the plasma insulin concentration (Fig. 7) Owing to the dehydration and circulatory failure in patients with ketoacidosis and with hyperosmolar coma subcutaneous injection of insulin is often insufficient as the absorption of insulin from the subcutaneous tissue is uncontrollable To be on the safe side very high doses of insulin have been given subcutaneously (up to 5000 i u /24 hours) This has entailed a risk of life-threatening hypopotassaemia hyperlactataemia and hypoglycaemia If the insulin therapy is given instead as intravenous injection hourly to every 4th hour there will be established an intermittent insulin deficiency state due to the very short biological half-life of insulin (cf p 209) These disadvantages may be avoided by continuous intravenous infusion of insulin 100 i u of short-acting insulin are dissolved in 1 litre of a physiological sodium chloride solution containing 0 1 % human albumin The albumin is added to avoid absorption of insulin to the infusion system If albumin cannot be procured the dose of insulin should be increased by about 25 % in order to compensate the adsorption of insulin to the infusion set (32 173) About 150 ml an hour is infused during the first 2 hours corresponding to 10-15 units/hour thereafter 50-100 ml/hour or 1 mU kg/min This form of treatment secures a constant and effective serum insulin level of about 80-100 μU/ml which lowers the blood glucose by 4-5 mmol/hour (91 151) If continuous intravenous infusion cannot be established intramuscular injection of insulin every hour 10 i u the first ? times and thereafter 2 5 i u /hour is the next best method (4) From the 2nd to the 3rd day of the treatment short-acting insulin should be injected subcutaneously 4 times daily for a few days thereafter intermediate-acting insulin Concerning fluid and electrolyte treatment consult reviews (81)

Institution of insulin treatment in diabetics without ketoacidosis

If the patients have glycosuria and ketonuria intensive observation is indicated either at home or in hospital If the general condition is poor the patient young febrile normal-weight or pregnant insulin treatment is always needed In obese patients in good general condition dietetic treatment may be tried first Insulin is administered subcutaneously as short-acting or intermediate-acting insulin in a dosage of about 0.2 to 0.5 i u./kg several times daily depending upon the general condition

If the patient does not have ketonuria it is justified before instituting insulin therapy to try other possibilities i e improving the endogenous insulin secretion increasing the insulin sensitivity and/or decreasing the insulin requirement. If acceptable metabolic control is not obtained (cf p 223) insulin therapy has to be instituted by an intermediate or long-acting insulin in a dose of about 0 to 0.3 i u /kg once daily The dose is gradually increased every 3rd to 4th day If acceptable metabolic control is not obtained on 35 to 40 i u in the morning insulin has to be given also in the evening

Continued insulin treatment

Most hospital units discharge their patients on too high a dose of insulin This is presumably because the patients are more active physically after returning home from the hospital and because complicating diseases which may have been responsible for a decreased insulin sensitivity are improving. It should be endeavoured therefore to secure as much physical activity during the stay in hospital as permitted by the general condition At the Steno Memorial Hospital this is possible Therefore the daily insulin dose for the out-patients is not lower than for the in-patients (Fig. 17) At all events the insulin treatment after discharge from hospital has to be adjusted by an out-patient follow-up In the case of beginners in insulin therapy the injected areas must be checked on this occasion and the injection technique discussed anew Not uncommonly the patients develop bad habits after their discharge from hospital For instance they may inject the insulin intracutaneously for fear of going too deep

or else they forget that insulin should be taken half an hour before a meal. If the insulin dose is to be altered, this must be done gradually, i.e. it may be altered 10 % to 15 % up or down, and then 3 days should elapse before further changes are made, unless acute situations require the doctor's help. Any changes should be made on the basis of the patient's condition (possible hypoglycaemia), body weight, postprandial blood sugar and urinary excretion of glucose. If the postprandial blood sugar is too high, short-acting insulin has to be combined with the intermediate- or long-acting insulin.

Insulin-treated diabetics should be seen quite often (every month to every 3 months), because many factors may influence the metabolic balance and because the patients may be feeling well for a long time, although the biochemical state is chaotic.

When is the follow-up examination satisfactory? In patients with maturity-onset diabetes we try to obtain and maintain:

- freedom from diabetic symptoms,
- freedom from hypoglycaemia,
- working capacity
- postprandial blood glucose concentration <11 mmol/l
- freedom from glycosuria,
- freedom from ketonuria,
- freedom from hyperlipoproteinaemia,
- normal body weight,
- acceptable blood pressure

In patients whose diabetes has set in before the age of 30 or other brittle diabetics, the above-mentioned demands concerning the postprandial blood sugar and glycosuria can generally not be fulfilled. In such patients we aim at postprandial blood glucose concentration below 15 mmol/l and a urinary excretion of glucose below 15 % of the 24-hour carbohydrate intake. In the case of children we add the demand of a satisfactory longitudinal growth and weight increase. Even though these demands are not

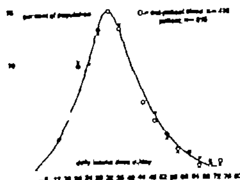


Fig. 17
Distribution of daily insulin requirement in 615 diabetic in-patients and 418 out-patients

fulfilled, the insulin dose need not always be altered. Slight changes in the distribution of carbohydrate intake over the 24 hours and/or a change in physical activity is often sufficient. Otherwise, the patients may easily be overdosed. At the Steno Memorial Hospital overdosage of insulin is one of the most common causes of a poor diabetes control in newly referred patients.

Discontinuation of insulin therapy

As already mentioned, many factors influence insulin requirements (cf. p. 215). These factors are not invariable in the same individual. Therefore, it may happen that a previously necessary insulin therapy can be discontinued. This is not infrequently possible in maturity-onset diabetics started on insulin therapy during a period of intercurrent disease or before a dietetic regime has given a result. The same applies to patients with pregnancy diabetes and young diabetics who go into remission (cf. p. 215). Resumption of the insulin therapy at a later date usually involves no risk of anaphylaxis to-day, when most insulins are of a high degree of purity. On the other hand, the development of insulin resistance (cf. p. 234) after intermittent insulin therapy is still a problem in many countries (56). It must, therefore, be recommenced to use insulins of an antigenicity as low as possible.

√*Insulin treatment combined with oral hypoglycaemic drugs*

Insulin therapy combined with oral antidiabetic agents of the biguanide type has been tried in juvenile diabetics *difficult to control* (121) but a long-term effect has not been obtained. Systematic investigations on insulin therapy combined with oral hypoglycaemic agents of the sulphonylurea type have not been performed and are probably not indicated.

3 Difficulties in insulin therapy

As stated on p 213 insulin treatment is an unsatisfactory substitution because in daily life it is not possible to administer the right dose at the right time or into the right place (into the portal vein) Therefore one must ease one s way along and coordinate the patient s life and insulin treatment by good and regular habits This demand for *regularity* is felt by many patients as the major handicap of diabetes Especially young people between 14 and 25 years of age can seldom live up to it as regularity in life comprises regularity of physical activity as well as of dietary habits and both are difficult to establish and observe during this period of life

That *dietetic indiscretions* and hyperphagia upset the metabolic balance is something all diabetics know

To these factors are added others affecting metabolic control and on which the patient can have no influence These are factors which lead either to changes in the serum insulin concentration or to changes in insulin sensitivity

Changes in the insulin concentration arise primarily as a result of erratic insulin absorption from the subcutaneous tissue By measuring the disappearance of radioactive Lente Insulin from the subcutaneous tissue in the same patient on different days Binder found a variation coefficient of 35 % (26) Also for porcine NPH-insulin the variation coefficient is considerable (Fig. 8) There is little doubt that if these findings indicate a variation in the absorption of insulin to the blood, this must be of the utmost significance to the daily *fluctuations in blood glucose* The insulin concentration may also vary owing to a dissociation of circulating insulin – insulin antibody complexes (60) In patients with insulin resistance severe insulin reactions have been observed several days after the discontinuation of insulin therapy Dixon et al (48) also found that insulin antibodies might influence the metabolic balance However the question concerning the role of insulin antibodies in the quality of control in insulin-treated diabetics is far from having been elucidated (cf p 238)

More gradually occurring changes in the insulin concentration may be seen when endogenous insulin production improves e g during the remission period (cf p 215) or when insulin elimination is affected as it is when severe hepatic and renal diseases develop

Changes in insulin sensitivity

Insulin therapy is often disturbed by rapid changes in insulin sensitivity The most common cause of such changes is the so-called Somogyi phenomenon i.e a hypoglycaemia-induced counter regulation (153) (Fig. 18) Not all diabetics can register and protect themselves from an abrupt fall of blood glucose or a fall of the blood glucose concentration to levels <3 mmol/l An abrupt fall in the blood glucose concentration or a fall of the blood glucose concentration to levels <3 mmol/l elicits a number of endocrine and metabolic processes whose integrated effect results in insulin antagonism The concentrations of growth hormone (161) and of noradrenaline (38) rise and in severe hypoglycaemic reactions also the concentrations of adrenaline (38) cortisol (16) and glucagon (15) However the increase in the glucagon concentration is often slight in insulin treated diabetics (15) All these hormonal reactions are most severe in pa

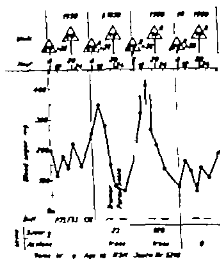


Fig 18
The Somogyi phenomenon
Δ[x] = NPH-insulin (international units)
Δ + 28 = NPH-insulin + 28 units of short-acting insulin.

tient with poorly controlled diabetes Regardless of whether the patients have had any symptoms of hypoglycaemia these hormonal reactions lead t a marked increase in blood glucose during the 24 hours following upon the hypoglycaemia. This so-called Somogyi phenomenon i often misinterpreted as sign of insulin deficiency but i in fact reaction to hyperinsulinism I ketosis-prone diabetics a sudden rise in blood-ketones can be seen following insulin-induced hypoglycemia – mostly likely due to an increase of the concentration of catecholamines (136, 142). Its treatment is easy when the real nature of the condition is realized.

Another common cause of rapid change in insulin sensitivity is febrile illness (65 138) and anxiety states (96) In these conditions too the concentration of insulin antagonistic hormones increases I certain countries ychological factors that may affect the insulin sensitivity are considered of such great importance that permanent psychological and psychiatric assistance has been established in the diabetes clinics (102). The large number of other factors which may more gradually affect the insulin sensitivity will not be discussed further here the majority are mentioned on p 215

Insulin treatment in surgical diseases

Minor surgery usually does not require any special precautions. Major surgery causes stress and is often accompanied by shifts in the electrolyte balance dehydration, and dyspepsia. These factors make the diabetes difficult to control. Reversely poor diabetes control may prove fateful burden to an operated patient (acidosis, dehydration, reduced resistance to wound infections and delayed wound healing). The microangiopathy and the frequently marked arteriosclerosis also increase the operative risk.

The following precautions should therefore be observed.In the case of non-acute operations the patient should for some days have been in good control, without ketonuria. On the morning of the operation 50 % of the usual morning dose in the form of an intermediate-acting insulin should be given subcutaneously Patients used to oral hypoglycaemic agents should be given 12 16 I.u. of an intermediate-acting insulin. Patients treated exclusively by dietary measures receive nothing. Immediately before the operation I litre of glucose 5 % for infusion with 32 I.u of short-acting insulin should be infused intravenously in the course of 3 hours This should be repeated after the operation, possibly supplemented by short-acting insulin 3 or 4 times daily depending upon the plasma glucose concentration and the ketonuria which should be determined 4 times daily If the patient is used to receiving an evening dose of insulin, this should as a rule be administered subcutaneously in the usual quantity

The precautions to be observed on the next day must depend upon the patient s condi-

tion The patient must have at least 150 g carbohydrate daily during the postoperative period and is then gradually changed over to *his normal diet and drugs*

In the case of acute surgery determination of the plasma glucose concentration standard bicarbonate serum potassium urinary glucose and ketones should be determined preoperatively The acidosis should be corrected by insulin, and a fall in the serum potassium concentration counteracted by the infusion of potassium-containing fluids As a rough rule it may be stated that at glucose concentrations exceeding 11 0 mmol/l 20 i u and at glucose concentrations exceeding 17 0 mmol/l 40 i u of a short-acting insulin should be administered subcutaneously before the operation In addition a glucose-insulin drip should be established as described above

Insulin treatment during pregnancy

During normal pregnancy the fasting plasma glucose level gradually decreases (128) If plasma glucose in pregnant diabetics cannot be kept within physiological limits by dietary treatment insulin treatment is indicated as the administration of sulphonylurea preparations may lead to dangerous hypoglycaemia in the newborn infant Besides the teratogenic effect of the oral hypoglycaemic agents has not yet been finally elucidated

The reason for the very strict demands on diabetes control during pregnancy is that the perinatal foetal mortality depends to a great extent upon the maternal diabetes control (53) In nearly all cases insulin has to be administered twice to several times daily (short-acting insulin plus intermediate-acting insulin) and it must be secured by weekly checking after the 20th week of gestation that *the insulin dose is being adequately adjusted*

Similarly good metabolic control around the time of conception is presumably of importance in avoiding abortion and congenital malformations It has been demonstrated by Yssing that infants of mothers regularly controlled in a specialized diabetological unit were born with fewer malformations than the infants of mother who did not attend for regular diabetes control (177)

Side effects of insulin therapy

As is apparent from Chapter I insulin is theoretically a well-defined polypeptide. However the insulin in the various marketed preparations is hardly as well-defined (cf p. 201). Thi is because insulin has to be solat ed from a pool of pancreatic proteins. Thereby it is not possible to avoid entirely contamination with insulin-foreign components from the pancreas Besides, n the course of purification clinical alterations of insulin and its precursor proinsulin may occur Owing to these factors, insulin therapy has been accompanied by a number of unpleasant local side effects – in particular previously when the protein-chemical methods were less advanced. To these are added disadvantages due to the physiological action of insulin as well as side effects related to the immunogenicity of the insulin preparations.

1 Disadvantages due to the physiological action of insulin

The most common untoward effect of insulin therapy is hypoglycaemic reactions The symptoms causes and effects of these reactions are described in many manuals. A few important factors concerning *hypoglycaemic reaction* will be mentioned here

Most diabetics feel an oncoming hypoglycaemia and react adequately If they have been correctly instructed (Fig. 19) The sensations may vary very much individually but as rule they are the same each time a patient has the insulin feeling"

There is little doubt that it is both the blood glucose level and its rate of decrease which elicit the nervous and hormonal reactions felt by the patient. Indeed, a patient s awareness of insulin feeling may change nature on changing from one insulin to another one with a new timing. In other patients – often elderly persons and patients with long-standing diabetes and/or patients with autonomic neuropathy – the warning symptoms of threatening hypoglycaemia may be entirely absent. These patients are in a very unfortunate situation, especially if their diabetes is difficult to control

Out of 309 patients who developed diabetes prior to 30 years of age between 1923 and 1933 and treated at the Steno Memorial Hospital, seven had died with hypoglycaemia before 1973

Medication with beta blocking agents may also eliminate or reduce the hypoglycaemic warnings. In insulin-treated patients alcohol intake may especially if not accompanied by intake of carbohydrate lead to serious insulin reactions partly because gluconeogenesis in the liver is reduced, partly because the warning symptoms are blurred. In well-controlled diabetics an "insulin feeling is not uncommon, mainly in connection with physical activity and before meals in patients treated with a morning dose of biphasic insulin as a rule about 11 12 noon (Fig. 19) The treat-

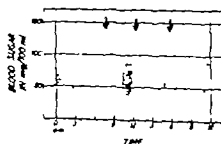


Fig 19
Blood sugar levels (Hagedorn Norman-Jensen) in 73 diabetics during 96 subjective sensations of hypoglycaemia ↓ = meal (By courtesy of V.H Asfeldt)

ment is rapid administration of glucose either by mouth or by the intravenous route Intramuscular injection of 0.5-1 0 mg glucagon may possible be used but especially in fasting children this treatment does not always succeed in raising the blood sugar After very severe and long lasting hypoglycaemic attacks unconsciousness may persist even after the blood glucose has been raised beyond 8 mmol/l In that case infusion of 100 mg hydrocortisone may be tried

Apart from hypoglycaemia a side effect which must be ascribed to an overdose of insulin there are other symptoms due directly or indirectly to the physiological actions of insulin but which feel unpleasant and may worry the patients because of their sudden onset These are transitory *refraction anomalies hypotension* and *oedema* The refraction disturbances are apt to occur in patients who are brought into a satisfactory metabolic balance after a period of poor control About the same applies to the so-called insulin oedema This occurs in predisposed individuals during the initial insulin therapy or when the insulin therapy is intensified in patients who have been poorly controlled throug a long time The pathogenesis has not been fully elucidated but is presumably a sudden and marked change in the ratio between the insulin and glucagon concentration in the plasma ratio of the utmost importance to the reabsorption of sodium in the renal tubules (14 93) The deposition of glycogen obtained by the insulin effect in the tissues formerly so poor in glycogen is also accompanied by retention of water

2 Local disadvantages

Among the local disadvantages of insulin therapy allergy will be discussed on p 23 In Denmark insulin allergy is extremely rare (cf p 236) but *lipodystrophy* is common i e insulin-induced changes of the subcutaneous tissue It is harmless and painless, but may constitute a severe cosmetic complaint It occurs as hypertrophy and/or atrophy of the subcutaneous fat (cf Figs 20 and 21) Hypertrophic changes are presumably due to the lipogenetic action of the injected insulin and are accordingly difficult to avoid entirely The local lipoatrophy on the other hand is probably due to impurities of the insulins At least it seems to have become considerably more rare after the advent of highly purified insulin preparations. Renold et al found an incidence of lipoatrophy of 24 ? % among insulin-treated diabetics – 44 4 % in patients under 20 years of age and 14 9 % in those who were over 20 (140) In the material from the Steno Memorial Hospital where 500 insulin-treated patients were examined consecutively lipoatrophy was found in only 1 8 % of the patients under 20 whereas the inciden



Fig 20
Hypertrophy of the fatty tissue after insulin injections for many years

e 10
uency of lipoatrophy in diabetics treated with insulins of different species and purity

...ment ...aration		n	Duration (years)	Lipoatrophy present (%)
...ine-bovine	NPH	341	16 1	16 4
...ine, monospecies	NPH	96	2.5	5.2
...ine high purified	NPH	51	5 5	1 9

...was 12 % in patients over 20 (43). The ...lanation of the low incidence of lipoat... ...y in the young patients and the high inci... ...ce in patients over 20 is presumably the ...er purity of modern insulin preparations. ...ed, many patients reported that the lipo... ...phies had developed during the first years ...e insulin therapy and that the areas of ...phy had previously been considerably ...e pronounced (Table 10).



21
...atrophy after insulin treatment in a pa-
... th diabetes mellitus

Korp and Levett (95) have also reported that lipoatrophy in insulin-treated patients might be reduced by the use of highly purified insu lin preparations. It is unlikely that the duration of the insulin therapy could explain the difference between the groups shown in Table 11 as in susceptible patients the lipoatrophy developed during the initial period of insulin therapy (171). The cause of the lipoatrophy is unknown. It is not likely to be a consequence of insulin antibody formation more probably of impurities in the insulins ...g. glucagon which is known to exert a lipolytic activity Another view is that the phe nomenon is due to neurovegetative disturbances in the area, entailing liberation of nor adrenaline and lipolysis.

3 Immunogenicity

There are 2 well-substantiated clinical manifestations of the immunogenicity of insulins Insulin allergy and insulin resistance Possibly other clinical phenomen (cf p 237) and the lipoatrophy (cf p 229) are related to it as well and it cannot be ruled out that late diabetic manifestations are aggravated by circulating insulin-insulin-antibody complexes

Cause of insulin antibody formation

In 1956 Berson and associates demonstrated circulating immunoglobulins that could bind insulin in insulin-treated patients (20) Apparently the insulin therapy was leading to antibody formation, and the insulin itself was the antigen. In Denmark circulating insulin

[illegible]

[illegible] The pathogenesis has not been fully [illegible] but is presumably a sudden and marked change in the ratio between the [illegible] and [illegible] concentration in the [illegible] of the [illegible] to the [illegible] absorption of sodium in the [illegible] (14, 15). The deposition of glycogen [illegible] by the insulin effect in the [illegible] formerly so poor in glycogen is also [illegible] by retention of water.

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Fig. 20
Hypertrophy of the fatty tissue after injections for many years

Table 10
Frequency of lipoatrophy in diabetics treated ith insulins of different species nd purity

Treatment Preparation			Duration (years)	Lipoatrophy present (%)
Porcine-bovine	NPH	341	16.1	16 4
Porcine, monospecies	NPH	96	2.5	5.2
Porcine high purified	NPH	51	1.5	1 9

ce was 12 % in patients over 20 (43) The explanation of the low incidence of lipoat rophy in the young patients and the high incidence in patients over 20 is presumably the higher purity of modern insulin preparations. Indeed, many patients reported that the lipoatrophies had developed during the first years of the insulin therapy and that the areas of atrophy had previously been considerably more pronounced (Table 10).



F g 21
Lipoatrophy after insulin treatment in pa- tient ith diabetes mellit

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antibodies were demonstrated in 97 % of in sulin treated patients whereas no insulin an tibodies could be demonstrated in non insu lin-treated diabetics or in normals (39)

In 1964 it was also reported that rabbits treat ed with impure porcine insulins were more likely to produce insulin-binding antibodies than were rabbits treated with more highly purified porcine insulins (31)

These findings gave rise to the following question Was the antigenicity of the insulins to man due to

(1) the species specificity of the insulin?
(2) the physical state of the insulins and the mode of application? and/or
(3) the degree of purity of the insulins i e their content of foreign proteins or insu lin-like modifications?

Species specificity

As already mentioned the primary structure of insulin differs somewhat from one animal species to another (cf Table 11) and it is well known that the primary structure of bovine insulin differs more from human insulin than does that of porcine and bovine insulin it is presumed that it was especially the content of bovine insulin which caused insulin antibody formation in insulin treated people That this may easily have been so is apparent from Fig 22 showing that patients treated

Table 11
The characteristic amino acids of insulin from different species

	A Chain			B Chain
Species	8	9	10	30
Human	Thr	Ser	Ileu	Thr
Pig	Thr	Ser	Ileu	Ala
Rabbit	Thr	Ser	Ileu	Ser
Ox	Ala	Ser	Val	Ala

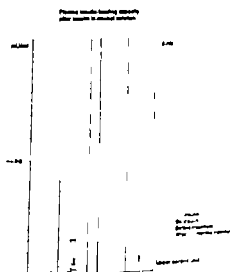


Fig 22
Plasma insulin-binding capacity in diabetics before and after treatment with insulin in neutral solution
( ) = Patient with allergic rhinitis (hay fever)
P = Patients treated with porcine insulin
O = Patients treated with bovine insulin
(from Andersen O O (9))

with pure bovine insulin in neutral solution develop considerably more insulin antibody than patients treated with pure porcine insu lin in neutral solution Similar findings have been made by others (22 44 54 101). Pending further studies therefore preparations containing bovine insulin must be considered more immunogenic than those containing porcine insulin Since 1968 practically all patients at the Steno Memorial Hospital have been treated with pure porcine insulin. Nevertheless insulin antibody formation could not be entirely avoided even in patients treated exclusively with NPH insulin made of porcine insulin In 75 % of these patients insulin antibodies were demonstr able after 6-9 months treatment i e at a time when insulin antibody format on i t maximum (7). The question remained whether the slight species difference between porcine and human insulin was the cause of the antigenicity of porcine insulin in man or

whether diabetics produce an insulin differing from normal human insulin and this was why the insulin therapy gave rise to antibody formation in diabetics

These questions were elucidated by iso-immunization experiments (30, 40 41) and studies of insulin extracted from the pancreas of deceased diabetics (33) However the investigations did not indicate that insulin extracted from the diabetic pancreas differed in structure biological activity immunological reactivity or composition of amino acid from insulin extracted from the non-diabetic pancreas (33) On the other hand the results of the isoimmunization experiments were remarkable Circulating insulin antibodies were demonstrable in pigs injected with porcine insulin without adjuvant (30 40) as well as in non-diabetic persons treated with recrystallized human insulin in neutral solution (41) It was therefore assumed that structural differences between porcine and human insulin could not be solely responsible for the antibody formation in people treated with porcine insulin Attention was thereupon directed at the other two questions Was the antibody formation due to the physical state

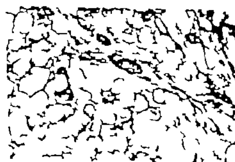


Fig 23
NSH 10856/65 18 years diabetes I ... I sulin treatment for on ear Biopsy of insulin-injected subcutaneous area 200 x PAS IIA Th ell are considerably in size Streaks of onnective tis ue elements and infiltrati ell are ob erved. Increased thick nes of essel all (A Werner NSH)



Fig 23 b
Same as Fig 23 a Closer view D tail 400 X Cellular infiltration consisting of eosinophil leucocyt s lymphocytes and histiocytes The thickened appearance of essel walls and fat cell membranes is obvious (A Werner NSH).

and mode of application of the insulin and/or to impurities of the insulin preparations having antigenic groups in common with insulin

Physical state

Insulin treatment of diabetics entails a concentration of insulin around the site of injection which is, immediately after the injection, about 10^4 times higher than the physiological concentration of insulin in the plasma. Moreover the daily injections cause recurrent injuries to the tissue leading to minor haemorrhages round-cell infiltrations and connective-tissue changes (Fig. 23). It cannot be ruled out that these factors play a role in the process of sensitization. That the physical tate of the insulin also plays a role is apparent from Fig. 24 The reason why only treatment with an acid insulin solution (9 40) or a neutral suspension of protamine insulin crystals (9) gives rise to antibody formation is presumably the following: After the injection of insulin dissolved at pH about 3 the organism will try to neutralize the injected solution. Thereby the iso-electric point of insulin is passed, and a precipitation takes place. As protein particles are assumed to be considerably stronger immunogens than protein mo-

antibodies were demonstrated in 97 % of insulin-treated patients whereas no insulin antibodies could be demonstrated in non-insulin-treated diabetics or in normals (39)

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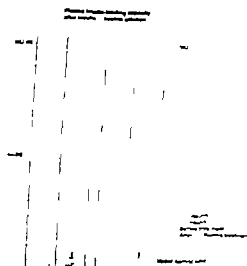


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Rabbit	Thr	Ser	Ileu	Ser
Ox	Ala	Ser	Val	Ala

whether diabetics produce an insulin differ ing from normal human insulin and this was why the insulin therapy gave rise to anti body formation in diabetics.

These questions were elucidated by iso-immunization e periments (30 40, 41) and studies of insulin extracted from the pancreas of deceased diabetics (33). However the investigations did not indicate that insulin e tracted from the diabetic pancreas differed in structure biological activity immunological reactivity or composition of amino acids from insulin tracted from the non-diabetic pancreas (33). On the other hand, the results of the isoimmunization periments were remarkable: Circulating insulin antibodies were demonstrable in pigs injected with porcine insulin without adjuvant (30 40) as well as in non-diabetic persons treated with re-crystallized human insulin in neutral solution (41). It was therefore assumed that structural differences between porcine and human insulin could not be solely responsible for the antibody formation in people treated with porcine insulin. Attention was thereupon directed at the other two questions. Was the antibody formation due to the physical state



Fig 23
NSH 10356/65 18 years diabetes 1 year I sulin treatment for one year. Biopsy of insulin-injected bcutaneous area 200 x PAS + HA The ell vary considerably in si. Streak of onnecti tissue element and infiltrati ell ar observed Increased thick es of sel all (A Werner NSH).



Fig 23 b
Same as Fig 23 a Closer view Detail 400 x Cell lar infiltration consisting of eosinophil l ucocyte lymphocytes and histiocytes The thickened ppearance of essel walls and fat cell membranes is obvious (A Werner NSH)

and mode of application of the insulin and/or to impurities of the insulin preparations having antigenic groups in common with insulin

Physical state

Insulin treatment of diabetics entails a concentration of insulin around the site of injection which is, immediately after the injection, about 10⁴ times higher than the physiological concentration of insulin in the plasma. Moreover the daily injections cause recurrent injuries to the tissue leading to minor haemorrhages round-cell infiltrations, and connective-tissue changes (Fig. 23). It cannot be ruled out that these factors play a role in the process of sensitization. That the physical state of the insulin also plays a role is apparent from Fig. 4 The reason why only treatment with an acid insulin solution (9 40) or a neutral suspension of protamine insulin crystals (9) gives rise to antibody formation is presumably the following: After the injection of insuli dissolved at pH about 3 the organism will try to neutralize the injected solution. Thereby the iso-electric point of insulin is passed, and a precipitation takes place. As protein particles are assumed to be considerably stronger immunogens than protein mo-

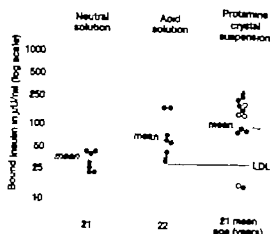


Fig 24
Plasma insulin-binding capacity in patients treated with porcine insulin for 180-270 days The same batch of insulin was used for all preparations
Insulin Leo Neutral® (neutral solution)
Insulin Leo® (acid solution)
Insulin Leo Retard® (suspension of crystalline protamine insulin)
LDL = lower detection limit for the plasma insulin-binding capacity in normal subjects and in diabetics not receiving insulin
** = patient with seasonal rhinitis (hay fever)*
Δ = examined before 180 days of treatment
o + examined after 270 days of treatment (from Andersen O O (9))

lecules in solution, the presence of minimal quantities of immunogenic substances in the injected insulins will be able to cause demonstrable antibody formation even though they are not capable of such formation when present in solution. This also applies to protamine insulins which are suspensions of protamine insulin crystals

Degree of purity

It has been found that even several times recrystallized insulin may contain traces of insulin-foreign components and several per cent of insulin modifications On gel filtration of crystalline insulin, which is not specially purified 3 fractions are demonstrable a b and c fraction (cf chapter 1) If insulin from more purified Scandinavian insulins is gel filtrated it is generally possible to demonstrate only 2 fractions b and c (cf Fig. 25) The a fraction presumably consists of insulin-foreign attendant proteins from the pancreas the b fraction of proinsulin (157) intermediary insulin and dimer insulin the c fraction of arginine insulin ethyl ester insulin insulin and insulin deamidated to a varying degree

That the b and c fractions consist of several components may be demonstrated by disc electrophoresis of the b fraction and c fraction (Fig. 26) From the immunological point of view the most important fraction of ordinary crystalline insulins is the a fraction and the b fraction, containing proinsulin (147). The explanation of the immunogenecity of the b fraction is that the difference in the C-peptide structure and thereby proinsulin between the various animal species is greater than the difference in their insulin structure (cf Chapter 2) Therefore in order to produce non-immunogenic porcine insulins of prolonged action it is important to purify recrystallized porcine insulin further so that at least the a fraction containing insulin-foreign components and the b fraction are removed (so-called single peak or monotop insulin)

The a fraction must be assumed to be highly antigenic to man It has been found that pro-

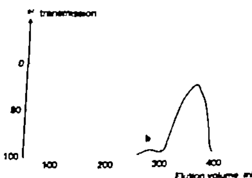


Fig 25
Fractionation of recrystallized porcine insulin on a Sephadex column The a- b- and c fractions are shown

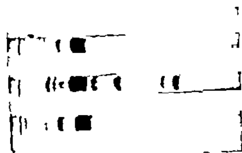


Fig 26
Disc electrophoresis of 100 μg crystalline porcine insulin (top) together with 100 μg b-fraction (middle) and 100 μg c-fraction (bottom) form gel filtration of crystalline porcine insulin



Fig 27
Plasma insulin-binding capacity in diabetic patients before and after treatment with NPH porcine insulin of ordinary quality (first 2 columns) and highly purified quality (last col mns) respectively

bably the cause of insulin antibody formation in persons treated with recrystallized human insulin in neutral solution was that these insulins contained small quantities of the a fraction (41). The reason why the a fraction – the one containing insulin-foreign components – gives rise to insuli antibody formation is presumably that this fraction contains proteins which have coupled to them insulin as haptens or which contain molecular structures having antigenic groups in common with insulin.

Whereas total removal of the proteins of the b fraction and of arginine insulin and ethyl ester insulin does not appear to be an absolute necessity for preventing insulin antibody formation in man treated with porcine insulin in neutral solution (cf Fig. 22), very careful purification of porcine insulin in order to remove these components is needed to reduce antibody formation when porcine insulins with prolonged action are used (Fig. 27).

Removal of these components is effected in various w ys resulting in a highly purified insulin also called "mono-component single-component or rare immunogenic (R.I.) insulin Gel filtration and disc electrophoresis of such highly purified insulin will show only one fraction (Fig. ?). However when a highly purified insulin is left to stand in solution, some of the insulin will gain be transformed, so that disc electrophoresis again shows several fractions after a few months (Fig. 28). In other words, it is possible to produce an insulin which apparently is a mono-component insulin immediately after the purification and under given conditions but in practice it is not possible to have mono-component preparations at disposal int. al. because of desamidation during storage However it must be considered extremely unlikely that the desamidation is of any importance to the immunogenicity of the insulin, as it has been demonstrated by Schlichtkrull et al. that injection of mono-component porcine insulin, even after storage for 2 years does not lead to antibody formation in rabbits (148).

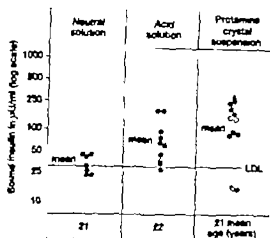


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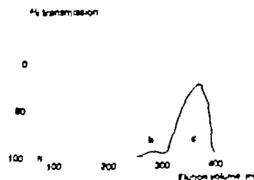


Fig 25
Fractionation of recrystallized porcine insulin on a Sephadex column The a- b- and c fractions are shown

this total insulin was assumed to be free insulin. However this could not be confirmed by Nakagawa et al. (129).

Animal experiments have revealed that immunization with insulin preparations may lead to insulitis a round-cell infiltration in and around the islets of Langerhans (109 141) and a diabetes-like state (72, 141). It cannot be excluded that similar factors apply in insulin-treated diabetics factors which must be assumed to be detrimental to the remaining B-cell mass.

Probably IgG antibodies can be deposited subendothelially in the retina, glomeruli and skin capillaries. At least IgG as well as insulin labelled with fluorescein isothiocyanate have been demonstrated in capillaries from patients with late diabetic manifestations (82).

Insulin antibodies are excreted in the urine with IgG globulins (39) and they pass the placenta (87).

In addition to circulating antibodies of the IgG type insulin antibodies of the IgM (143) and IgE type exist (58). The latter are responsible for insulin allergy (58). Furthermore specific antibodies to proinsulin have been demonstrated in insulin-treated patients (110).

Clinical significance of insulin antibodies

Insulin antibodies play a role in the development of insulin allergy and insulin resistance. Furthermore they seem to influence also the amount of the daily dose. On the other hand, their influence upon the duration of remission, the stability of diabetes control, lipoatrophy and the development of late diabetic complications is more controversial.

Insulin allergy

Immediately after the introduction of insulin therapy it was obvious that injection of insulin preparations might give rise to allergic reactions (86). However these reactions have grown less common with the increasing purity of insulin preparations (85) and in patients treated from the very beginning of insulin therapy with highly purified preparations allergy has so far not been seen (43). It must be assumed, therefore that the insulin allergy is not induced by insulin, but by polypeptides contaminating the insulin preparations. However once the patients are sensitized, even highly purified insulin may occasionally give rise to local allergic reactions (57 63). There is no correlation between circulating insulin antibodies of the IgG type and allergic reactions (39) but between antibodies of the IgE type and insulin allergy (58). As highly purified insulin preparations are not yet in general use in all parts of the world insulin allergy is still a therapeutic problem in some places.

Insulin allergy manifests itself either locally as red spots around the sites of injection or systemically as urticaria or anaphylactic reactions. The local reactions around the sites of injection are most common. They are of the delayed hypersensitivity type and usually appear about one week after the institution of insulin therapy The erythema sets in 4-24 hours after the injection, measures from 1 10 cm in diameter and may be accompanied by itching and burning. As a rule the skin is slightly swollen and warm, but indolent. If the cutaneous changes are small, they usually subside within 24 hours and are not registered by the patient. This form of allergy generally subsides spontaneously in the course of a few weeks during continued insulin therapy but may crop up later

Whereas insulin allergy of the delayed hypersensitivity type occurs at the institution of insulin therapy insulin allergy of the immediate type may occur at any time during the treatment. It manifests itself as red spots frequently also of urticarial nature around the site of injection immediately or up to hours after the injection. This reaction too is

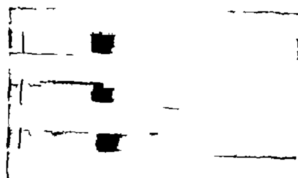


Fig 28
Disc electrophoresis of 100 µg highly purified porcine insulin and of 100 µg highly purified porcine insulin which has been stored for 3 months at 4°C (middle) and 25°C (bottom) respectively

That careful purification of porcine insulin is of importance to the immunogenicity of the long acting insulins has been demonstrated by a number of workers (11 12 34 43 55 95 105 119 147) Fig. 27 gives the results of a study in the Steno Memorial Hospital The concentration of insulin antibody was measured as the plasma insulin binding capacity by the method of Ortved Andersen (6) Insulin antibody formation was studied before and after 12 24 months treatment with a highly purified NPH porcine insulin preparation This insulin was characterized by showing after gel filtration only one peak on acrylamide gel electrophoresis only insulin and desamido insulin and on radioimmunological determination of proinsulin to have a proinsulin content of less than 0 5 mmol/mol insulin It will be seen that only 4 out of 55 patients had formed demonstrable quantities of insulin antibodies after 24 months of treatment.

Insulin antibodies

Thus to-day it is possible to produce from porcine insulin long-acting preparations having little or no immunogenicity This is not yet possible using bovine insulin (16) Since however bovine insulin has to be used because of the inadequate supply of porcine insulin it must be anticipated that insulin antibody formation cannot be completely avoided The cause of the immunogenicity of bovine insulin has not been definitely elucidated Perhaps bovine insulin cannot be purified as effectively as porcine insulin but it cannot be ruled out that bovine insulin *per se* is responsible owing to a structure differing more from human insulin

Insulin antibodies of the IgG type are demonstrable 2-4 weeks after the institution of insulin therapy The titres reach a maximum in 6-9 months and then fall slowly (Fig 29). Young patients show more marked antibody formation than older patients (7) and intermittent insulin therapy results in a very pronounced antibody response (40 63).

Once the insulin antibodies have formed their specificity to the insulin is generally low as they can bind insulins of porcine bovine as well as human origin (71).

It is doubtful whether the concentration of free insulin rises in step with the development of insulin antibodies Heding demonstrated that the total insulin concentration in the serum rose markedly at the time of insulin antibody formation (79) A considerable part of



Fig 29
Insulin-binding capacity of plasma tested before and during treatment with insulin The dotted line indicates the lower detection limit for the antibody determination
(from Andersen O O (7))

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But red spots around the sites of injection do not always indicate an allergic cutaneous reaction They may be due to an incorrect (intracutaneous) technique of injection (43) To secure a diagnosis of insulin allergy an intracutaneous test with 0 1 ml of a 1 10 dilution of the preparation concerned must show a definitely positive reaction as compared with a control injection of the solvent without insulin If there is a suspicion of systemic hypersensitivity lower doses of insulin should be used for the intracutanous test In insulin allergy of the delayed hypersensitivity type the skin reaction must be positive in 6-24 hours in skin reactions of the at once type in 5-60 min after the injection

In Denmark insulin allergy is rare At the Steno Memorial Hospital insulin allergy was diagnosed during the period 1951 1963 in 0.5 % of almost 3 000 insulin-treated patients (39) From 1963 1973 insulin allergy was diagnosed only once (6 971 admissions) This patient was a foreigner who had developed the allergy while using a non Scandinavian insulin preparation.

In a systematic study of the injection sites in 500 consecutive patients treated with intermediate insulins red spots exceeding 5 mm in diameter were found ½-3 hours after the injection in 1 0 % In another 2 cases the positive reaction proved to have been caused by the injection on the day or evening before the day of the study These patients had been on insulin therapy for 9 to 14 years Intracutanous testing was not performed In all cases the reaction was asymptomatic and had not been noticed by the patients. In all cases the red spots had disappeared 2-4 months later but in 2 of the cases not until the injection technique had been corrected (43)

From other countries the incidence of local cutaneous reactions is reported to be considerably higher (56) but data comparable with the values stated above have not been published

Systemic allergic reactions are extremely rare and only 8 cases of anaphylactic shock are on record (77 118)

If the patient has insulin allergy with trouble-some allergic symptoms it should be investigated, whether the symptoms can be overcome by changing to another insulin preparation Attention should be directed at the species specificity and degree of purity of the insulin preparations The pH content of desinfectants and retarding substances are seldom of any importance (56) Indeed allergy to protamine (70 90) has never been reported and to Surfen * rarely (100) In practice this means that the patients must be tested with highly purified insulin preparations from different species first with short-acting insulin and thereafter with the same insulin prepared as long-acting (63) In the Anglo-Saxon countries preparations containing insulin modified in different ways (desalanine insulin (98) sulphated insulin (124) and maleyl insulin (125)) may be tried If testing with highly purified insulins also induces a highly positive reaction the patient has to be desensitized if he cannot do without insulin therapy Antihistamines are of no use and cortisone is unnecessary The desensitization process is carried out by a short acting highly purified porcine insulin The procedure is started by the subcutaneous application of dose which gives rise to no or only slight complaints on intracutaneous injection Daily the dose is gradually increased until the therapeutic dose is reached Thereafter the treatment may be changed to long acting insulins of the same species and some degree of purity

Insulin resistance

Previously insulin resistance was defined as a state in non ketoacidotic, insulin-treated persons whose daily dose of insulin had to

exceed 200 i.u. to produce a clinical effect (113). This definition is out-of-date now since the mean daily dose of insulin is around 36 i.u. There is an increasing tendency to apply the term insulin resistance to cases in which the dose of insulin exceeds 100 i.u./24 hours for several consecutive days. Not infrequently insulin-resistant patients require several thousand units of insulin daily. The highest dose of insulin ever used is 177,500 units/24 hours (166). In spite of the high dose of insulin the effect is often minimal and several patients succumb in a state of gradually developing ketoacidosis.

The causes of insulin resistance are manifold partially unknown, but one of the most common causes is an excessive binding of insulin to circulating IgG insulin antibodies (56). Often insulin resistance co-exists with insulin allergy (56). To confirm the diagnosis an insulin sensitivity test must be done in non-allergic patients, using 0.1 unit of short-acting insulin per kg body weight injected intravenously and recording the blood glucose every 10 minutes. Normally a distinct fall of the blood glucose occurs within 30 min. Moreover the serum must be studied for insulin antibodies. In immunologically conditioned insulin resistance the insulin binding capacity of the serum is very high, but there may be exceptions. If the insulin sensitivity test fails to induce a fall of blood glucose and insulin antibodies are demonstrable the patient has immunologically conditioned insulin resistance.

In Denmark immunologically conditioned insulin resistance is rare. At the Steno Memorial Hospital there has not been one case of immunologically induced insulin resistance (24-hour dose of insulin >100 i.u. + insulin antibodies) in the past 5 years. On the other hand it may be mentioned that 3.6 % of Federal patients in West Germany have an insulin requirement exceeding 100 i.u./24 hours (56). Life-threatening insulin resistance is extremely rare.

[illegible]

Fig. 30
Daily insulin needs in two groups of patients. The first group of patients was treated with the usual NPH porcine insulin, the other with highly purified NPH porcine insulin
(from Deckert et al. (43))

The treatment consists in lowering the insulin requirement (calorie restriction), raising the insulin sensitivity (exercise, psychotherapy etc.), and increasing the endogenous insulin production as far as possible (sulphonylurea preparations, etc.). If these measures prove insufficient, the insulin preparation should be replaced with a highly purified porcine insulin or a modified insulin (2, 106). Possibly immunosuppression may be tried using cortisone acetate and cyclophosphamide, or azathioprine in immunosuppressive doses. If immunosuppressive therapy is successful its effect will be manifest during one week. Otherwise, it should be discontinued. During these various therapeutic attempts the patient should be treated with continuous intravenous infusion of insulin. If the patient is allergic desensitization must be instituted without delay before higher doses of insulin are given.

Daily dose of insulin

There is no doubt about the positive correlation between insulin antibody titre and the daily dose of insulin in patients whose daily dose is high (56). However "normal" insulin antibody titres also seem to influence the daily dose (cf. Fig. 30). When switching over from treatment with highly antigenic insulins to less antigenic insulins, the patient must be prepared to reduce insulin dosage (126). This must be done in particular when changing from bovine insulin to highly purified porcine insulin (13). Whether the daily dose decrea-

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If the patient has insulin allergy with trouble-some allergic symptoms, it should be investigated whether the symptoms can be overcome by changing to another insulin preparation Attention should be directed at the species specificity and degree of purity of the insulin preparations. The pH content of desinfectants and retarding substances are seldom of any importance (56). Indeed allergy to protamine (70 90) has never been reported and to Surfen ® rarely (100). In practice this means that the patients must be tested with highly purified insulin preparations from different species first with short-acting insulin and thereafter with the same insulin prepared as long-acting (63) In the Anglo-Saxon countries preparations containing insulin modified in different ways (desalanine insulin (98) sulphated insulin (1 4) and maleyl insulin (1.5)) may be tried If testing with highly purified insulins also induces a highly positive reaction the patient has to be desensitized if he cannot do without insulin therapy Antihistamines are of no use and cortisone is unnecessary The desensitization process is carried out by a short-acting, highly purified porcine insulin. The procedure is started by the subcutaneous application of dose which gives rise to no or only slight complaints on intracutaneous injection Daily the dose is gradually increased until the therapeutic dose is reached Thereafter the treatment may be changed to long acting insulins of the same species and some degree of purity

Insulin resistance

Previously insulin resistance was defined as a state in non-ketoacidotic insulin-treated persons whose daily dose of insulin had t

exceed 200 i.u to produce a clinical effect (113). This definition is out-of-date now since the mean daily dose of insulin is around 36 i.u There is an increasing tendency to apply the term insulin resistance to cases in which the dose of insulin exceeds 100 i.u./24 hours for several consecutive days. Not infrequently insulin-resistant patients require several thousand units of insulin daily The highest dose of insulin ever used is 177,500 units/24 hours (166). In spite of the high dose of insulin the effect is often minimal and several patients succumb in a state of gradually developing ketoacidosis.

The causes of insulin resistance are manifold partially unknown, but one of the most common causes is an excessive binding of insulin to circulating IgG insulin antibodies (56). Often insulin resistance co-exists with insulin allergy (56) To confirm the diagnosis an insulin sensitivity test must be done, in non-allergic patients, using 0.1 unit of short-acting insulin per kg body weight injected intravenously and recording the blood glucose every 10 minutes. Normally distinct fall of the blood glucose occurs within 30 min. Moreover the serum must be studied for insulin antibodies In immunologically conditioned insulin resistance the insulin binding capacity of the serum is very high but there may be exception If the insulin sensitivity test fail to induce fall of blood glucose and insulin antibodies are demonstrable the patient has immunologically conditioned insulin resistance

In Denmark immunologically conditioned insulin resistance is rare At the Steno Memorial Hospital there ha not been one case of immunologically induced insulin resistance (24-hour dose of insulin >100 i.u. + insulin antibodies) in the past 25 years On the other hand it may be mentioned that 3.6 % of Federal patients in West Germany have an insulin requirement exceeding 100 i.u./24 hours (56) Life-threatening insulin resistance is extremely rare

Fig 30

Daily i sulin need in two groups of patients The first group of patient wa treated with the u ual NPH porcine insulin the other with highly purified NPH porcine insulin

(from Deckert et al (43)

The treatment consists in lowering the insulin requirement (calorie restriction) raising the insulin sensitivity (exercise psychotherapy etc) and increasing the endogenous insulin production as far as possible (sulphonylurea preparations, etc.) If these measures prove insufficient the insulin preparation should be replaced with a highly purified porcine insulin or a modified insulin (., 108) Possibly immunosuppression may be tried using cortisone acetate and cyclophosphamide or azathioprine in immunosuppressive doses. If immunosuppressive therapy is successful its effect will be manifest during one week. Otherwise, it should be discontinued During these various therapeutic attempts the patient should be treated with continuous intravenous infusion of insulin. If the patient is allergic desensitization must be instituted without delay befor higher doses of insulin are given.

Daily dose of insulin

There is no doubt about the positive correlation between insulin antibody titre and the daily dose of insulin in patients whose daily dose is high (56) However "normal insulin antibody titres also seem to influence the daily dose (cf Fig. 30) When switching over from treatment with highly antigenic insulins to less antigenic insulins, the patient must be prepared to educe insulin dosage (126) This must be done, in particular when changing from bovine insulin to highly purified porcine insulin (13) Whether the daily dose decrea-

often devoid of symptoms but may spread on the skin and manifest itself as a transition to generalized allergic reactions This form of allergy too may often subside spontaneously

But red spots around the sites of injection do not always indicate an allergic cutaneous reaction They may be due to an incorrect (intracutaneous) technique of injection (43). To secure a diagnosis of insulin allergy an intracutaneous test, with 0 1 ml of a 1 10 dilution of the preparation concerned must show a definitely positive reaction as compared with a control injection of the solvent without insulin If there is a suspicion of systemic hypersensitivity lower doses of insulin should be used for the intracutanous test. In insulin allergy of the delayed hypersensitivity type the skin reaction must be positive in 6-24 hours In skin reactions of the "at once type in 5-60 min after the injection.

In Denmark insulin allergy is rare At the Steno Memorial Hospital insulin allergy was diagnosed during the period 1951 1963 in 0.5 % of almost 3 000 insulin-treated patients (39) From 1963-1973 insulin allergy was diagnosed only once (6 971 admissions) This patient was a foreigner who had developed the allergy while using a non Scandinavian insulin preparation.

In a systematic study of the injection sites in 500 consecutive patients treated with intermediate insulins red spots exceeding 5 mm in diameter were found ½-3 hours after the injection in 1 0 % In another 2 cases the positive reaction proved to have been caused by the injection on the day or evening before the day of the study These patients had been on insulin therapy for 9 to 14 years Intracutanous testing was not performed In all cases the reaction was asymptomatic and had not been noticed by the patients In all cases the red spots had disappeared 2-4 months later but in 2 of the cases not until the injection technique had been corrected (43)

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ses when the treatment is changed from a non-highly purified porcine insulin to a highly purified porcine insulin has not been definitely elucidated Presumably the reduction in the dose is slight involving no risk to the patient, as the biological half life of circulating insulin antibodies is several weeks and as the antibody titre in patients treated with porcine insulins is low (7)

Period of remission

That insulin antibodies presumably influence the length of the remission period is apparent from a study by Ortved Andersen (8) who followed 22 consecutive insulin treated diabetics during the period of remission after insulin therapy had been initiated After a follow-up period of one year only 4 of the 10 patients who had formed insulin antibodies were still in remission whereas 8 of the 12 patients who did not form insulin antibodies were still in remission The explanation of the influence exerted by the insulin antibodies upon the length of the remission period is presumably that circulating insulin antibodies are able to bind and eliminate endogenous insulin (72) and thereby lead to a more rapid exhaustion of the patients remaining B-cell mass Moreover, an immunological damage to the B-cells cannot be ruled out

Diabetes control

Whether the control of diabetes is facilitated in the sense of fewer blood glucose fluctuations on highly purified porcine insulin preparations cannot be decided with certainty Ortved Andersen demonstrated that the quality of the diabetes control in young diabetics is significantly correlated to the insulin-binding capacity in the serum but this did not apply to elderly patients (7) Dixon et al (48) made the opposite finding viz. that patients with an unstable metabolic balance had small amounts of insulin antibodies or insulin antibodies of high avidity whereas patients in stable metabolic balance had larger quantities of insulin antibodies of low avidity in the serum

Late diabetic manifestations

It is uncertain whether circulating dissolved insulin-insulin antibody complexes play a role in the development of late diabetic manifestations Wehner et al (172) found changes in the basement membrane of glomerular capillaries from mice treated with immunogenic insulin preparations but not in mice treated with insulin preparations of low immunogenicity However these findings could not be confirmed by Hägg (82) It must be mentioned also that labelled insulin (137) as well as immunoglobulins have been demonstrated in the vascular wall of insulin treated diabetics (103) It is doubtful however whether these findings are to be attributed with pathogenetic significance

Let it be mentioned at last that non-immunogenic insulin preparations may be of importance also in research In patients who do not form insulin antibodies it has proved possible to follow the insulin secretion also during insulin therapy Previously this could not be done because of insulin antibody formation

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A New Swedish Twin Registry

containing environmental and medical base line data from about 14 000 same-sexed pairs born 1926 – 58

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SUMMARY

Since the beginning of the 1960 s the Department of Environmental Hygiene of the Karolinska Institute and of the National Swedish Environment Protection Board has maintained a registry on about 10 000 same-sexed twin pairs born 1886-1925 The registry has been used for studies of morbidity and mortality against the background of certain external risk factors especially smoking

Supported by the Research Committee of the National Swedish Environment Protection Board, the establishment of a new twin registry covering younger age cohorts was initiated in 1970 The main purposes of this registry are to evaluate individuals adaptation to changes in the environment and to study the effects of the environment on human health Moreover the registry is intended to be usable in obtaining specific target groups with a certain exposure

The main reason for the establishment of a twin registry instead of using a representative sample from the entire population is that a twin population offers some additional analytical possibilities With twins composing the study group it is possible to assess the role of the genetic factor for different va iables In addition the twin approach provides opportunities of analyzing the subjects as matched pairs the twin control method This method involves the evaluation of the effect of one factor to which one twin in the pair is exposed and the other not while other variables are kept constant in a far-reaching way e g sex age genetic composition child hood environment etc

A twin registry can also be employed as a general epidemiological base line registry For such purposes the twin methodology is ignored and the effects of some agent for example having to do with environmental hygiene are studied on all of the individuals independently of their twin pair qualities The registry is then in many regards comparable with a registry of non twins

The first step in creating the Swedish twin registry was to compile information on names and addresses By means of searches in birth records for the years 1926-1949 and birth notices for the years 1950-1967 registration was made of ca 110 000 twin individuals which constitute virtually all twins accounted for n official statistics Of these ca 13 800 were stillbirths or infant deaths Before the search for the twins current addresses was begun all pairs in which at least one individual was dead were eliminated Such was the case in ca 10 250 pairs The address search hence covered about 89 500 indi iduals and was carried out with the assistance of two location procedures The first was applied to those born between 1926 and 1949 and the second to those born between 1950 and 1967 The result was that in ca 96% of the pairs both members (37 590 pairs) or one member (4 853 pairs) could be found It could be anticipated that of the latter pairs 1 450 would not be useable due to the death of one of the twins

The data collection has been carried out via a mail questionnaire which consists of the following main areas of inquiry: medical symptoms and use of medication annoyance experienced because of factors in the general and occupational environments smoking and drinking habits physical activity food habits psychosocial status occupational and educational history conditions of place of dwelling and certain background data

INTRODUCTION

In the early 1960 s a comprehensive epidemiological research program was launched at the Department of Hygiene the Karolinska Institute and the affiliated Department of Environmental Hygiene at the then existing National Institute of Public Health The program called for studies of morbidity and mortality as associated with certain external risk factors particularly smoking The studies were subsequently carried out upon twins in a registry compiled by the departments and encompassing ca 10 000 same sexed pairs born between 1886 1925 Particular attention was devoted to the effects of environmental agents upon groups of persons with similar constitution and also to studies elucidating the role of the hereditary factor in diseases and behavioral traits

The Research Committee of the Swedish National Environment Protection Board promulgated this epidemiological research program in 1970 by supporting the creation of a new twin registry The new registry consists of over 26 500 same-sexed twin pairs born between 1926 and 1967 A questionnaire was sent to twin pairs born between 1926 and 1958 ca 21 000 pairs Responses were received from ca 32 400 individuals which included a little over 13 900 complete pairs

The coming decades are anticipated to experience essential changes in both the outer and inner environment the exact manifestation of which can only be conjectured Factors which point to negative changes are intense industrialization greater concentrations of population in already densely populated areas more automobilism and increased traffic in general Positive changes can be expected due to environmental legislation monitoring and research

One purpose behind the registry is to evaluate individuals adaptation to this kind of changes in the environment Such studies presupposes a longitudinal follow-up with renewed contacts with the twins after a number of years At present however effects of the environment on human health in a broad sense including annoyance reactions as well as medical symptoms can be studied using cross sectional data already available

The information in the registry could also be used to select specific target groups for example type of exposure at work or in the general environment One might also wish to find persons with certain medical or social characteristics Examples of target groups are persons with different residential or occupational characteristics Another target group could be vulnerable individuals who because of special social or somatic disorders run a increased risk of environment-related health impairment

It is often motivated to perform sporadic investigations to illuminate the effect of some environmental factor for example odor soot or dust on populations living in the vicinity of industries or the effect of noise or air poll tion on populations living near airports and freeways A new sample for each such occasion would entail quite great costs Furthermore the exposure can only be evaluated in retrospect for short periods of time e g because of the subjects difficulties in recalling it The twin registry has been designed to offer complementary material for such investigations The difficulties which arose when the medical significance of eating mercury contaminated fish was to be studied can serve as an illustration The most urgent need was obviously to examine individuals ith a very heavy consumption of fish Since these are relatively few

Before the questionnaire was sent out to the twin population it was tested in a pilot investigation which purported to study the effectiveness of the data collection procedure and to elucidate the validity of some of the individual questions The pilot investigation showed an external nonresponse rate of 18% Regarding the ability of the subjects to answer the questions it was revealed that only a few of the question categories presented problems which gave rise to minor changes in the questionnaire Comparison between the answers on the questionnaire and a parallel interview showed a high degree of agreement Thus the questionnaire was considered satis factory

The principal data collection was carried out during the period January May 1973 and during the period December 1973 - February 1974

Of the 42 294 individuals included in the data collection 3 339 could not be contacted because of death illness etc Responses have been received from 32 374 individuals which corresponds to 83% of the subjects who could be contacted Regarded as a population of twin pairs 3 262 pairs have not been utilizable for similar reasons The total number of pairs in which both have answered the questionnaire is 13 811 corresponding to a little over 77%

There are three versions of the twin registry a birth registry an address registry and a questionnaire registry The birth registry contains birth data from about 110 000 twin individuals The address registry contains in addition current information as to name and address as well as current and past census registration The questionnaire registry which comprises twins born 1926 1958 moreover contains information obtained on the basis of the mail questionnaire

BACKGROUND AND BASIC CONCEPTS

Motives behind the twin registry

The motivation for setting up a population registry consisting of twins instead of a random sample from the Swedish population is that twins offer analytical possibilities beyond those of the other type of sample If the study is purely descriptive and aims at estimating parameters in the population a random sample should generally be preferable Twins are in certain regards not completely representative of the general population They have for example a specific prenatal environment It is known that twins compared with the general population show a higher infant mortality rate and a lower birth weight

The problem of representativeness has been discussed by Cederlöf (1966) who nonetheless could not discern any striking differences between twin and singletons with regard to such variables as number of siblings number of children education marital status place of residence smoking behavior and symptoms of lung or heart impairment Moreover twins do not appear to deviate from the general population with regard to relative cancer mortality (Harvald and Hauge 1968 Cederlöf et al 1970)

A twin population enables an evaluation of the role of the genetic factor in the emergence of special conditions and qualities Certain hereditary and environmental questions primarily in the fields of psychology and psychiatry have been studied in twins since the end of the 1800 s These studies have usually been based upon relatively few twin pairs not randomly chosen but because one or both of them display the particular quality under study (selected twins) The analytical method employed in these connections is generally termed the classical twin method A brief account of the methods for evaluating the genetic factor will be presented below

Another advantage afforded by twins is that they may be analyzed as matched pairs in which one individual is exposed to some agent and the other is not while a series of other variables automatically are kept under control This method is usually referred to as the twin control method In a series consisting of monozygotic pairs the twins not only have the same sex and age but in addition have identical genetic constitutions As regards same-se ed dizygotic pairs, sex and age are again equivalent and genetic factors are on an average 50% the same Regardless of zygosity twins most often have shared the same environment in childhood and adolescence and tend to have comparable experiences in later life as well The control of se and age and the genetic comparability should be of special importance in studies of diseases or somatic symptoms and their etiology Epidemiological research began to utilize this twin methodology around 1960 The analytical models will be schematically described below

E isting registries of non selected twins

Major regist ies of non-selected twin pairs have been set up in Scandinavia and in the United States One of the first registries was compiled around 1950 by Essen Möller and consisted of twins living in Skåne the southern part of Sweden This twin series has been utilized in a number of studies mainly related to psychiatric and psychosomatic problems (Essen-Möller 1963 Et rha d 1968)

perhaps 10% of the population the mere sorting out of these individuals would call for a comprehensive screening procedure Had data on dietery habits been stored in a registry let us say on 25 000 individuals a computerized procedure could have sorted out a sufficient number of individuals quite rapidly

The new registry has been developed during four years The main stress in the following will be on the data obtained on matters of interest for environmental medicine In giving such an account however it has been considered essential to provide a relatively detailed description of the compilation procedure behind the creation of the registry Such a description is well motivated since twin registries are being set up in other countries The experiences undergone during the collection of the material for the new Swedish twin registry should also be of value for the setting up of registries of non twin populations

This project could never have been accomplished without the cooperation of various administrative authorities organizations and individuals Financial support has been provided by the National Swedish Environment Protection Board the National Swedish Food Administration and the Council for Tobacco Research U S A

Lennart Danielson chief inspector at the Swedish Product Control Board of the National Swedish Environment Protection Board and Vera Sandahl at the Department of Statistical Services of the Central Bureau of Statistics have given us much appreciated constructive criticism and advice

We gratefully acknowledge the able assistance of the staff at the Depart ment of Environmental Hygiene at the National Swedish Environment Protection Board Mary Jern has borne the responsibility for the manual routines The major part of the key punch operation has been carried out by Gun Andersson Monica Hedlund and Ann-Margret Lindevall Other persons who have played a substantial role in the project are Lena Bergström Gun Inger Loboda Eva Britt Gustafsson and Eva Undsén Pamela Boston has translated the Swedish manuscript into English

The willingness of the twins to fill in the questionnaires has of course been the sine qua non for this program

This report was originally published in Swedish in the beginning of 1976 by the National Swedish Environment Portection Board (Report series PM 670)

These registries together with the registry to be described in the following as the new Swedish twin registry" are at present fairly unique In other countries efforts have been made to establish similar twin registries Finland will within a few years have completed a twin registry of the same type as the Swedish one

Methods of analysis

Evaluation of a genetic dependence

Several kinds of hypotheses can be set up with regard to heredity and environment and the methods of evaluation are equally diverse Different theoretical models have previously been summarized (Twin Registries in the Study of Chronic Disease 1971)

The classical twin method calls for a comparison between monozygotic and dizygotic twin pairs If the monozygotic pairs in some regard show similarity to a significantly higher degree than the dizygotic pairs this is considered to stem from genetic factors and the variable in question is concluded to be genetically dependent The method assumes that the individuals within the monozygotic pairs are exposed to similar environmental agents to the same extent as individuals within the dizygotic pairs This assumption however is notably not entirely valid (Ustlyngen 1949)

The classical twin method entails the analysis of concordant and discordant pairs i e pairs in which one or both twins show a particular trait These concepts are illustrated in Figure 1 The degree of similarity for each zygosity group can be calculated in two different ways These are usually designated as pairwise concordance rate and proband concordance rate and have been discussed in more detail by Allen et al (1967) The pairwise concordance rate refers to the number of concordant pairs in relation to the total number of pairs in which one or both twins are positive with regard to the trait being studied According to Figure 1 then the pairwise concordance rate would be 33% for monozygotes and 11% for dizygotes The proband concordance rate consists of the number of positive individuals with a positive partner in relation to the total number of positive individuals which in Figure 1 would be 50% and 20% for monozygotes and dizygotes respectively

The rate of concordance may be more or less adequate depending on which variable is being studied For example if the analysis concerns a disease which occurs late in life it is improbable that both members of a pair would develop the disease at the same time The older the subjects are the greater becomes the probability of concordance Hence if monozygotes and dizygotes are to be compared the age distributions in the two groups must also be equivalent

The signficance of the genetic factor can also be evaluated by calculating the so-called coincidence for the two zygosity groups This approach assumes that the number of pairs in which both members are free from the trait in question is known in line with what is seen from the example in Figure 2 Here the coincidence i e the number of positively concordant pairs in relation to all observed pairs is 10% for monozygotes and 4% for dizygotes This type of data offers the possibility of relating the coincidence values to the probability that both members of a pair will show a likeness In the example a total of 60 positive cases occur among 300 observed individuals giving a prevalence of 20% Under the assumption that no genetic dependence exists for the given factor

The Danish twin registry

In Denmark a registration of twins was begun in 1954 and has continued into the present The registry encompasses same sexed twin pairs born in Denmark between 1870 and 1920 provided that both twins within a pair lived to be at least five years of age For the 1870 1910 age cohorts the registry comprises both same- and opposite sexed pairs The compilation procedure has been described by Hauge et al (1968) The registry mainly contains medical data and the principal aim has been to elucidate the role of the genetic factor in the development of various diseases e g cancer and coronary heart disease (Harvald and Hauge 1963 1970) Epidemiological studies concerning the association between tobacco smoking and health have also been carried out (Hauge et al 1970)

The older Swedish twin registry

The older Swedish twin registry was compiled during 1959 1961 and covers ca 10 000 pairs It includes about 95% of all same sexed pairs born within the country between 1886 and 1925 provided that both members of the pair were alive at the time that the registry was set up A detailed description of the compilation procedures and demographic data has been given by Cederlöf (1966)

The main purpose of the registry has been to study morbidity and mortality against the background of various exposure factors Studies performed to date have primarily concerned diseases of the respiratory system such as cough and chronic bronchitis coronary heart disease and cancer The exposure factor given the foremost attention has been tobacco smoking (Cederlöf et al 1966 Cederlöf et al 1970 Friberg et al 1970 1973) Some of the other exposure factors treated are air pollutants (Cederlöf 1966) alcohol consumption (Friberg et al 1973) and psychosocial factors (Floderus 1974) Studies concerning the role of hereditary factors in the emergence of certain diseases have also been made (Cederlöf et al 1967a 1967b 1970)

The data have been collected mainly via three large mail questionnaires and through matching with official registries The major epidemiological investi gations based on the questionnaire data and concerning the registry as a whole have been supplement with clinical investigations on sub-samples (Lundman 1966 Liljefors 1970 Myrhed 1974 de Faire 1974)

The U S twin registry

The U S twin registry is a sample taken from a twin material stored at the National Academy of Sciences in Washington which in turn has been taken from the Veterans Administration Files It is composed of white male twins born in 39 states during the years 1917 1927 and serving in the U S Armed Forces This twin series numbering ca 4 000 pairs has been presented by Jablon et al (1967)

One of the epidemiological investigations performed on the U S registry has a resemblance to those carried out on the older Swedish twin registry (Cederlöf et al 1969 Hrubec et al 1973) Thus it has been possible to verify certain research results on an independent material The major part of the questionnaire data in the older Swedish twin registry has been equivalently collected in the U S registry

These registries together with the registry to be described in the following as the "new Swedish twin registry" are at present fairly unique In other countries efforts have been made to establish similar twin registries Finland will within a few years have completed a twin registry of the same type as the Swedish one

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Figure 1   Study group subjected to calculation of concordance an example

	Number of pairs	
	MZ	DZ
CONCORDANCE	5	4
DISCORDANCE	10	32
Total	15	36

Figure 2   Study group subjected to calculation of coincidence an example

	Number of pairs	
	MZ	DZ
POSITIVE CONCORDANCE	5	4
DISCORDANCE	10	32
NEGATIVE CONCORDANCE	35	64
Total	50	100

the probability calculation gives that 100 (0 2 x 0 2) = 4% of the pairs would be positively concordant through pure chance The difference in coincidence between monozygotes and dizygotes is assumed to reflect the influence of the genetic factor In the dizygotes the difference between expected coincidence and observed coincidence to a great extent can be assumed to express similarities between the two twin individuals with regard to environment This difference can sometimes be considerable The table below shows some coincidence values from material presented in this report and concerning men in the age cohort 1946 1958

	MZ		DZ	
	Expected %	Observed %	Expected %	Observed %
Allergy	2 7	9 5	2 8	4 9
Back trouble	1 4	4 6	1 4	2 5
Worked over-time	2 7	7 9	2 7	5 6

The observed coincidence among the dizygotes is about twice as great as the expected while that among the monozygotes is about 3 times as great

B-series analysis

What is commonly denoted as the twin control method has been referred to as B series analysis in investigations centering upon the older Swedish twin registry This method of analysis calls for a selection of twin pairs discordant with regard to some independent variable which the investigator intends to relate to a dependent variable One example would be to sort out smoking discordant pairs i e pairs who differ as to smoking habits The analysis would then focus upon to what extent these pairs also display a difference with regard to a dependent variable such as illness As has been mentioned this method allows a relationship to be analyzed while variables such as age sex genetic factors and childhood environment are held constant

Figure 3 illustrates the B series analysis approach All smoking discordant pairs are shown divided into disease concordant pairs (positive or negative concordance) and disease discordant pairs As a rule only disease discordant pairs are used in the statistical analysis The proportion of sick smokers with healthy non smoking partners is weighed against the proportion of healthy smokers with sick non smoking partners in this case 35 35 among monozygotes and 45 25 among dizygotes An approriate test of significance in this context is the McNemar test for the significance of changes (Siegel 1956) In certain cases pairs with positive disease concordance can be used in the statistical analysis provided that information as to onset of illness (or date of death) is available

If one in the above example is studying a variable such as body weight which can be measured with high precision the evaluation may of course be made by means of a parametric method such as a common t test for dependent samples

A series analysis

In earlier publications concerning the Swedish twin registry the so-called A-series analysis have been presented This form of analysis treats the data on all twin individuals entirely independently of their pair charac teristics The material is then regarded a random sample of the population There are several reasons for handling the data this way

In the great majority of cases twins should not deviate from individuals in the total population Thus A-series analysis in many respects may provide opportunities of estimating the prevalences and degree of association in the total population i e the A series analysis gives information in the same manner as any other population sample

The A-series analysis offers a contrast to the twin based analysis and furnishes information on the relationship (e g dose-responses) between groups of unrelated individuals It is worth noting that the A-series analysis performed upon the Swedish twin registry to a great extent verifies the excess morbidity in smokers documented in a large number of epidemiological studies The methods for collecting information on smoking as well as on morbidity may hence be concluded to be sufficiently valid the twin material as such may be inferred not to differ from a random sample of the population in any measureable respect In the event that the B series analysis does not point to the same relationships as does the A-series analysis the method cannot therewith be deemed insensitive Instead this disagreement should chiefly depend upon genetic or environment matching effects

The sample on which the A-series analysis is carried out may consist of either one twin from each pair or both twins in each pair In the former case a series of statistically unrelated individuals is obtained In the latter case, the pair-wise relationships of the individuals obviously affect the statistical analysis As long as the concordance is moderate this dependence should be possible to disregard Otherwise a correction for this concordance must be performed during the statistical analysis

The question of the representativeness of the twin population for the total population has been discussed earlier in this chapter It should be noted that point estimates may be somewhat biased due to lack of representativeness At the same time it should be emphasized that studies of dose-response associaton between variables should not be noticably affected by this circumstance

Figure 3 Study group subjected to B series analysis an example

	Smoker	Non smoker	Number of pairs MZ	Number of pairs DZ
POSITIVE CONCORDANCE			10	6
DISCORDANCE			35	45
			35	25
NEGATIVE CONCORDANCE			120	124
Total			200	200

A series analysis

In earlier publications concerning the Swedish twin registry the so-called A-series analysis have been presented This form of analysis treats the data on all twin individuals entirely independently of their pair characteristics The material is then regarded a random sample of the population There are several reasons for handling the data this way

In the great majority of cases twins should not deviate from individuals in the total population Thus A-series analysis in many respects may provide opportunities of estimating the prevalences and degree of association in the total population i e the A series analysis gives information in the same manner as any other population sample

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COMPILATION OF BASE DATA

The first step in the compilation of the twin registry was to obtain the names and addresses of all twins born between 1926 and 1967 provided that both members of a pair were residing in Sweden This procedure was divided into two stages first the registration of all twin births during the period in question and secondly the locating of the twin with regard to current name and address

Registration of twin births

Information on twin births between 1926 and 1949 was obtained via a manual search through birth records on file at the Central Bureau of Statistics (SCB) These records are divided into volumes according to year of birth Within each volume the births are subdivided into county municipality and parish The information on each birth includes date of birth first name surname sex marital status of mother live or still birth and parish Multiple births have a specific denotation

Information on twin births between 1950 and 1967 was obtained by manual scanning of special cards so called birth notices which are also on file at SCB The cards organized according to the same principle as the birth records also include the same information with one important addition the birth notices give the entire population census code number (year + month + day of birth + 4 code numbers assigned to that individual alone) Multiple births are again given a special designation

Since multiple births other than twin births could be obtained more or less automatically and were considered to be of potential value these were also registered Multiple births in which one or more of the members were registered as dead were also included in the registry

The registration task commenced in June 1970 continued througout the summer and autumn On the average ca 150 multiple births were registered per day by one task worker The work was subjected to a continuous check to ensure that all births were registered and that the information noted was complete

The information was key punched and verified during the autumn and winter of 1971 which resulted in almost 55 000 punch cards each pertaining to a single pair The punch cards were transferred to a magnetic tape edited and thoroughly scrutinized

Locating the twins

To find the current addresses names and the like computerized comparisons were made between the compiled twin registry and a registry of the total Swedish population the RTB registry which contains data on all persons residing in Sweden

Since complete population census code numbers were available for the twins born between 1950 and 1967 the so-called ID-routine developed by the Central Bureau of Statistics could be used to find the addresses of these twins The ID-routine was run in February 1971 Addresses were obtained for 34 308 persons out of the 36 114 sought

Twins born prior to 1950 were located by sorting out potential twins from the RTB-registry The twins were coupled via a manual examination to individuals selected from the RTB registry The selection was made by matching date and county of birth as well as sex County of birth is not stated explicitly in the RTB registry but each county has its own series of birth numbers

A twin was considered to be identified in this manual comparison procedure if at least one of the following two critera were fulfilled

1 Date of birth sex county of birth first name and surname are in accord

2 Date of birth sex county of birth and first name are in accord for a complete twin-pair and these two individuals have birth numbers near each other in the RTB-registry

Twins are generally assigned consecutive birth numbers but even numbers are reserved for girls and odd ones for boys This practice explains the leeway allowed by the second criterion in near each other

The selection from the RTB registry resulted in ca 550 000 individuals who filled the requirements The manual matching was performed in the summer of 1971 by five persons About 90% of the twins could be located in this manner

This location procedure was impaired by certain systematic errors The population census code number system was introduced in 1947 At that time persons were assigned numbers from a numerical series reserved for the county in which they were then residing This circumstance meant that older persons could not be located as easily as younger once Moreover twins who had changed their names as through marriages and twins delivered with a large interval of time were more difficult to identify For these reasons a new sorting out of names from the RTB-registry was undertaken This time the criterion for selection was agreement as to birth data and sex

Res lts

Informat on concerning the completeness of the twin registry is summarized in Tables 1 3 Description of the material with regard to different variables is given in the section on the content of the data

Birth registry

Table 1 shows i a the number of twins born and the number registrered during the manual scanning of the birth records and birth notices It can be observed that more births than those reflected in the official statistics have sometimes been registered This most likely is due to errors in the official statistics A person born during a certain year may have been entered into the birth records of the proceeding year The official statistics set up on an annual basis take up only the persons entered into the birth records during the year to which the statistics pertain

Table 1 also reveals differences in the results depending upon the source material For the period 1926-1949 the percentage of twins found by using the

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Table 1 Number of twins according to public statistics and the number of twins found in birth records and birth notices
Breakdown according to year of birth

Year of birth	Birth according to official statistics[x]			Registered out of birth records and birth notices					
	individuals	pairs	stillborn individuals	individuals	pairs	%[xx]	died in infancy	individuals in living pairs	living pairs
1926 1929	11 608	5 804	933	11 626	5 813	100 2	1 848	8 936	4 468
1930-1934	12 482	6 241	1 025	12 486	6 243	100 0	1 932	9 616	4,808
1935-1939	12 466	6 233	991	12 442	6 221	99 8	1 976	9 534	4 767
1940-1944	14 938	7 469	932	14 980	7 490	100 3	2 220	11 756	5 878
1945-1949	16 034	8 017	919	16 040	8 020	100 0	1 804	13 316	6 658
1950-1954	12 810	6 405	675	12 730	6 365	99 4	1 327	10 704	5 352
1955 1959	11 362	5 681	482	11 374	5 687	100 1	1 149	9 608	4 804
1960-1964	11 480	5 740	396	11 216	5 608	97 7	1 021	9 708	4 854
1965-1967	6 956	3 478	240	6 886	3 443	99 0	542	6 094	3 047
Total	110 136	55 068	6 493	109 780	54 890	99 7	13 819	82 272	44 636

x) Sources: SOS population movement 1925-1960 SOS shifts in population 1961 66 SOS changes in population 1967
SOS The Swedish Government Official Statistics

xx) Calculated as the number of registered individuals in percent of the numbers of individuals in the official statistics

birth records is a little over 100% while the percentage of twins found by using the birth notices is about 99% Since the birth notices are filing cards they can easily become mislaid or lost The Central Bureau of Statistics has worked intensively with the birth notices in their project to computerize birth statistics It is possible that not all of the cards were refiled afterward For instance certain cartons with notices from 1967 were missing

The number registered as dead in the birth records and birth notices greatly exceeds the number of still births in the official statistics as is evident from the table This stems from the fact that the notation of death in the birth records and birth notices refers to whether or not the individual died before his birth had been entered upon a birth record or notice

Address registry

Since the object was to create a registry of twins in intact pairs all pairs in which one or both members were dead were removed from the material consisting of twins to be located The number of pairs then remaining is seen in Table 3

To judge the effectiveness of the locating procedures an estimation of the number of twins expected to be alive and residing in Sweden in 1971 was needed In such cases mortality is unrivaled as a decimating factor Since the exact meaning of dead in infancy" was unknown a mortality prognosis was made assuming that all persons not registered as dead survived their entire first year of life The prognosis shown in Table 2 should therefore underestimate the true mortality

Table 3 shows the results of the locating procedures broken down into pairs in which both have been located one has been located and neither has been located In addition the expected number of living pairs is illustrated divided in the same manner

When the earlier twin registry encompassing the birth records for the years 1886 1925 was being compiled a complete pair was considered located as soon as one partner had been found since it was possible to ask the known partner to fill in the unknown partner s address in the subsequent mail questionnaire Table 3 also shows the expected number of pairs useable due to location of both members

A random sample of 50 non located twins born in 1926 was subjected to further scrutiny These were traced from birth through contacts with the county boards and parish registrars offices The results yielded 44 deaths 3 emigrated twins and 3 twins residing in Sweden This check to some extent vouched for the effectiveness of the locating procedure

Table 3 Located expected number of survlv ng among the located and useable twin pairs

Year of birth	Total number of living twin pairs	Location successful			Expected nr living 1971			Useable pairs (both living in 1971)		
		Both	One	Neither	Both loc	One loc	Neither loc	Total	At least one located	percent
1926-1929	4 468	2 905	1 111	452	2 905	694	268	3 867	3 599	93 1
1930-1934	4 808	3 442	991	375	3 442	673	245	4 360	4 115	94 4
1935 1939	4 767	3 780	751	236	3 780	515	158	4 453	4 296	96 5
1940-1944	5 878	5 047	678	153	5 047	457	102	5 606	5 504	98 2
1945-1949	6 658	5 595	656	407	5 595	533	330	6 458	6 128	94 9
1950-1954	5 352	4 899	295	158	4 899	226	123	5 248	5 125	97 7
1955 1959	4 804	4 456	189	159	4 456	152	127	4 735	4 608	97 4
1960-1964	4 854	4 581	105	168	4 581	87	138	4 806	4 668	97 2
1965-1967	3 047	2 885	77	85	2 885	64	71	3 020	2 949	97 7
Total	44 636	37 590	4 853	2 193	37 590	3 401	1 362	42 553	40 991	96 4

Table 2 Number of twins alive in 1971 according to prognosis x)

Year of birth	Pairs of same-sexed men: Registered number of living pairs	Prognosis 1971: Both alive	One dead	Both dead	Pairs of same-sexed women: Registered number of living pairs	Prognosis 1971: Both alive	One dead	Both dead	Opposite sexed pairs: Registered number of living pairs	Prognosis 1971: Both alive	One dead	Both dead	Total: Registered number of living pairs	Prognosis 1971: Both alive	One dead	Both dead
1926 1929	1 366	1 165	193	8	1 363	1 197	161	5	1 739	1 505	226	8	4 468	3 867	580	21
1930 1934	1 447	1 286	156	5	1 542	1 425	115	2	1 819	1 649	166	4	4 808	4 360	437	11
1935 1939	1 484	1 364	117	3	1 463	1 389	73	1	1 820	1 700	118	2	4 767	4 453	308	6
1940-1944	1 852	1 744	106	2	1 874	1 809	64	1	2 152	2 053	98	1	5 878	5 606	268	4
1945-1949	2 901	2 030	78	1	2 065	2 018	47	0	2 484	2 410	74	0	6 658	6 458	199	1
1950-1954	1 746	1 707	39	0	1 604	1 578	26	0	2 002	1 963	39	0	5 352	5 248	104	0
1955-1959	1 576	1 553	23	0	1 474	1 453	21	0	1 754	1 729	25	0	4 804	4 735	69	0
1960-1964	1 576	1 560	16	0	1 534	1 519	15	0	1 744	1 727	17	0	4 854	4 806	48	0
1965 1967	1 026	1 016	10	0	988	980	8	0	1 033	1 024	9	0	3 047	3 020	27	0
Total	14 182	13 425	738	19	13 907	13 368	530	9	16 547	15 760	772	15	44 636	42 553	2 040	43

x) Source Statistiska Meddelanden (Statistics Communication) Be 1970 3 Kohortdödligheten i Sverige (Cohort mortality in Sweden)

Table 3 Located expected number of surviving among the located and useable twin pairs

Year of birth	Total number of living twin pairs	Location successful			Expected nr living 1971			Useable pairs (both living in 1971)		
		Both	One	Neither	Both loc	One loc	Neither loc	Total	At least one located	percent
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Total	44 636	37 590	4 853	2 193	37 590	3 401	1 362	42 553	40 991	96 4

INSTRUMENTS OF MEASUREMENT

Base date were collected via a mail questionnaire with the following main areas of inquiry medical symptoms and use of pharmaceuticals annoyance felt toward agents in the general and occupational environments smoking and drinking habits physical activity dietary habits psychosocial status occupational and educational background dwelling conditions and certain background data The questionnaire embraces both dose and response variables Whether a given variable should be regarded as dose or response depends on the hypothesis in question Smoking can be analyzed as a dose variable in association with respiratory symptoms but it can also be analyzed as an individual s response to a psychosocial disturbance A headache can be an effect of annoyance due to noise disturbance and at the same time a cause for taking pharmaceuticals

It has been necessary to treat the wide spectrum of variables represented in the questionnaire in varying depths Certain questions, such as those on dietary habits are designed primarily to supply criteria for sorting out risk groups Other questions such as those on drinking habits are intended to serve for fairly rough classifications (high" versus low consumers) and divisions into type (beer wine or hard liquor consumers) Still other questions for instance those on smoking aim at providing relatively detailed information In some cases the questions consist of generally accepted diagnostic criteria or scale indices as for example "angina pectoris and "psychosocial instability"

Areas of inquiry

The medical data primarily concern specific symptoms of the heart and respiratory system The battery of questions worked out by Rose (1962) in order to diagnose angina pectoris has been included This method has previously been the object of special validity studies (Cederlöf et al 1966 Lundman et al 1971) Heart and lung status have been treated as effect variables in connection with e g smoking and alcohol consumption in a number of studies based on the older twin registry (Cederlöf et al 1966 Lundman 1966 Liljefors 1969 Friberg et al 1973 and Myrhed 1974) Headache, hearing impairment as well as stomach back and nervous problems are essential effects va iables in studies on environmental impact be it general or occupational Questions concerning these symptoms have been included in the questionnaire and the phrasing to a large extent has followed the principles employed in the health status control program of the Swedish Foundation for Industrial Safety and Health in the Construction Industry (Östlund 1974) In addit on an open question has been posed in which the respondent may describe any diseases of a long-lasting or serious nature which he or she has suffered

A relevant approach in assessing a subject s somatic as well as mental health status is to inquire into the consumption of common pharmaceuticals It would also have been of value to obtain information on intake of narcotics However since the likelihood of obtaining valid data was considered small such questions were not posed This subject might instead be focused on special investigations in the future

A major object of the questionnaire has been to create a basis for detecting and evaluating annoyance evoked by agents in the occupational and residential

The intake of various foods in interaction with other environmental factors is of considerable importance for the individual s health status (Arvidsson et al 1973) A complete description of dietary habits would be beyond the scope of this investigation only limited aspects have been studied Questions on dietary habits have been directed toward the consumption of food products and basic foodstuffs which may be suspected to bring risks for example in the form of heavy metals (organ foods fish shellfish) The frequency of warm meals in contrast to snacks such as sandwiches and coffee is also investigated This aspect is expected to covary with psychological as well as somatic variables

A covariation between psychosocial factors and somatic disease has been observed in a number of studies (Caffrey 1967 Jenkings 1971) An association has also been noted between psychosocial status and risk factors such as smoking drinking and decreased physical exercise during leisure-time (Floderus 1974) The psychosocial variable might then be interpreted as a cause behind these traits And individual s psychosocial status can probably influcence the way he experiences environmental agents such as noise and air pollution Conversely it may be logical to analyze psychosocial disturbances as being effects of e g annoying agents in the living and occupational environments A psychosocial classification has thus been considered essential and accordingly a series of questions from the Eysenck s Personality Inventory has been selected and tested (Floderus 1974)

Certain conventional background variables such as sex age family housing education and occupation have been covered in the questionnaire Not only questions about the occupational category but also the nature of the work and the amount of overtime shift-work piece-work and extra work as well as previous employments were included With regard to housing the type and size of dwelling number of persons living together and adjustments in the particular place of residence have been focused on In addition the person was asked about his geographical mobility

Questions on the target population s twinship concerned contact frequency as well as the time they lived together and zygosity Zygosity is determined by asking the twins whether they were like two peas in a pod or not more alike than siblings in general during their childhood and adolescence This formulation has repeatedly proven to be of high validity when substantiation via serological tests has been carried out (Cederlöf et al 1961 Lundman 1966 Jablon et al 1967 Myrhed 1974) The twinship questions were not included in the questionnaire for the pilot investigation since it was directed toward a random sample of subjects

Pilot investigatio

The questionnaire was pretested prior to its being sent out to the twin population The purpose was to find out whether the respondents could comprehend the questions correctly and could evaluate the alternative responses Moreover the lay-out of the questionnaire and the respondent s ability to follow directions were tested Technical aspects of data collection and external non-response were also studied

The pilot investigation was carried out in two phases In phase I a mail questionnaire investigation was made and in phase II a limited number of personal interviews were performed on a subsample

environments Objective environmental data on e g aircraft noise in the vicinity of the respondent s home should be obtainable from administrative or other sources The questionnaire has addressed itself to the subjective perception of environmental agents In other words to what extent did the respondent notice or not notice potentially annoying agents and to what extent did he experience these agents as annoying? Annoyance reactions towards aircraft noise have been examined specifically in a number of studies (Sörensen 1970 Rylander et al 1972)

It would also have been valuable to elucidate manifestations of annoyance i e under what circumstances the annoyance appears and what kind of effects that are evoked in terms of awakening inability to sleep etc A pronouncedly high association between such manifestations and a general annoyance reaction has previously been reported (Arvidsson et al 1968 Sörensen et al 1973) Against this background and the limited space available it was considered sufficient for the present purpose to include only a few questions measuring more general annoyance reactions

Smoking habits must be surveyed not only to study the possible harmful effects of smoking but also to provide a control variable when the effects of various environmental agents are to be investigated Both quantitative and qualitative aspects of the exposure to tobacco should be taken into account The quantitative aspect is the amount of tobacco per unit of time as well as exposure time while the qualitative aspect is the type of tobacco The questionnaire is designed to give both a current and a retrospective picture of smoking habits as to both type and quantity Mail questionnaires have been demonstrated to provide a relatively good validity with regard to tobacco consumption (Cederlöf Jonsson 1965) This matter has undergone extended analysis in a smoking study at the Department of Environmental Hygiene at the Karolinska Institute (Cederlöf et al 1975) The technique of inquiry in the questionnaire at hand is not entirely identical to that used earlier but there is no reason to believe that validity would be affected therewith

Previous twin investigations have revealed an association between consumption of alcohol and somatic variables such as physiological and metabolic parameters and increased mortality (Friberg et al 1973 Myrhed 1974) Alcohol then is a risk factor which should be essential as a pure dose variable but also as a control variable in the study of other dose-response relationships Subjective statements about drinking habits are generally considered to be encumbered with systematic errors Results from studies comparing the metho dology of mailed questionnaires and personal interviews suggest that the questionnaire method if anything gives data of a higher validity as compared with the interview technique (Björkman 1971)

Another factor of relevance in an individual s health status is physical activity (Manelis et al 1969 Cassel et al 1971 Arvidsson et al 1973) Adequate measures such as average caloric expenditure are naturally impossible to collect in a mailed questionnaire Instead rather rough ordinal rank orders of the individuals have to be relied upon The questionnaire contains questions about the degree of physically exertive versus sedentary work as well as the amount of physical exertion during leisure time The model for these questions has been the questionnaire used by the National Board of Health and Welfare in Sweden to investigate diet and exercise among the Swedish people (Arvidsson et al 1973) Some reduction in the number of questions has been made to suit the questionnaire at hand

Table 4 The sample and the external non-response in the mail questionnaire investigation

	Total		Tringsund		Varberg	
	n	%	n	%	n	%
Sample size	1192		596		596	
Answers submitted	975	82	489	82	486	82
Non-response						
Refusals	10	1	4	1	6	1
Not possible to trace	9	1	2	0	7	1
Serious illness	2	0	2	0	0	0
Others	196	16	99	17	97	16

With regard to internal non response the final result is dependent upon the intensity with which the missing information is gathered Normally questions considered to be of particular interest are used as the criterion for whether or not a follow-up is necessary A subject with whom a new contact is made according to this policy is asked to complete any questions which he answered incompletely though these questions in themselves would not have been criteria for follow-up It is difficult after follow-up to regard the non-response frequency of an individual question as an indicator of its degree of difficulty or of the respondent s willingness to answer it Instead the residual drop-out should be judged as the end effect of the follow-up procedure

Certain main questions regarding e g angina pectoris respiratory symptoms and smoking and drinking habits have here been employed as follow-up criteria as well as the entire battery of questions of annoyance Of the questionnaires submitted 31% were returned to the respondent for additional information after which the majority of the individual questions turned out to have an internal non-response of 0-3% In some batteries a large drop-out frequency remained even after follow-up This was true of e g certain details in the questions on annoyance

Agreement between mail questionnaire and personal interview

Below some comparisons between mail questionnaire and personal interview are presented This material is by no means claimed to serve as a validation of the questions For one thing the data are too limited for such an interpretation for another both methods are fetterred with relatively large errors compared with a true criterias e g clinical diagnoses Concepts such as sensitivity and specificity have therefore been avoided Even the agreement found should be interpreted cautiously since the possibility of dependence between methods in the form of memory effects or changes from one occasion of investigation to another cannot be entirely excluded

Groups investigated

Two sampling areas were chosen namely a suburb of Stockholm (Trångsund) and a fairly large city in the southwestern part of Sweden (Varberg) In each area a random sample of 600 persons born 1927 1957 was selected Eight persons of the total of 1 200 were no longer living in the area which brought the defined sample size to 1 192 individuals The non response rate will be discussed under the heading Results since this was one of the main problems of the pilot study

When the sample for the questionnaire investigation had been constituted 100 individuals from each geographical area were randomly selected to partici pate in the subsequent interview investigation Interviews were carried out on 91% of the persons who did fill in the questionnaire and on 44% of the persons who did not

Method

It should be emphasized that the aim of the pilot investigation was not to compare questionnaire versus interview method The interview purported to provide a more in-depth elucidation of the question complexes and to study differences in information as well as their causes These findings enabled an improvement of the questionnaire

The interview was restricted to cover the most important areas of inquiry of the mail questionnaire In some cases the questions were identical to those of the questionnaire in other cases they were expressed either as unstructured questions or as structured questions taken from more comprehensive and often used instruments

In certain cases e g in the battery of questions on medical symptoms the interviewer knew how the respondent had answered the mail questionnaire In the event of a discrepancy the respondent was asked to explain Even when agreement was found ther interviewer had to assure himself that the reponse was adequate e g that the respondent actually gave all symptoms or illnesses

Results

Non-response

The non response of questionnaire studies includes the external as well as internal drop-out External non response rerfers to failure to return the form as such despite reminders to do so while internal non response refers to incomplete responses to the different questions The sample and the external non response which amounted to 18% in each geographical area are seen in Table 4

could not be distinguished from non reponse of other origin The response alternatives were revised so that persons who did not consider themselves exposed to a given annoying agent could indicate this Morevoer construc tional changes in the questions enabled even persons with a low dregree of annoyance to continue to the subsequent questions concerning frequency and intensity of annoyance The annoyance battery was given more space in the questionnaire

Questions on the most annoying environmental agent were treated as follows an index was constructed which weighed together the intensity and the frequency of the annoyance as stated in the personal interview responses whereupon the most annoying environmental agent was established for each individual The result was compared with the responses on the mail questionnaire where the most annoying agent had been stated directly It was found that 96% of the subjects reported the same agent on the two occasions with respect to the home environment With regard to agents in the occupational environment the agreement was 93%

Smoking habits As mentioned previously a study of the validity of questions on smoking habits has been carried out earlier The methodology of that study also called for a comparison of data obtained via a mail questionnaire and those obtained via a personal interview The agreement found in that study was considered good Despite this fact it was considered essential to reanalyze the present manner of posing questions The greatest interest was attached to cigarette smoking consumption age at the debut and age at cessation The results are shown in Table 6 Non-smokers are also included in the table which increases the degree of agreement

Table 6 Cigarette consumption agreement

	Agreement	
	Difference at most 4 cigarettes or at most 2 years	Difference at most 5 cigarettes or at most 3 years
Number of cigarettes/day	81%	92%
Age at debut	86%	96%
Age at cessation	84%	93%

Agreement as to quantity of cigarettes is good provided a deviation of 5 cigarettes is accepted The ages at debut and cessation also show a fairly stable response pattern

Table 7 shows the agreement between the personal interview and the mail questionnaire regarding smoking history

Medical data Certain of the questions concerning medical data were based on those previously used by the Swedish Foundation for Industrial Safety and Health in the Construction Industry Their modified versions used here have not been subjected to testing Comparisons between mail question naire and personal interview responses are shown in Table 5 The negative responses in the interview were countered by but a few positive responses on the questionnaire while the positive responses in the interview were met with less of an agreement

The interview indicated that the questions on use of pharmaceuticals had shortcomings These did not stem from difficulties in defining the drugs as such but from the response alternatives on consumption frequency In the questionnaire the alternative never was provided Upon closer inquiry in the personal interview it came forth that numerous respondents did use a given drug but virtually never   This held true primarily with regard to non prescription painkilling drugs The response alternative was changed in accordance with this finding

Table 5 Medical data Agreement in absolut numbers

Interview	Pos	Pos	Neg	Neg
Questionnaire	Pos	Neg	Pos	Neg
Shortness of breath	23	4	9	104
Severe chest pain (30 min)	4	2	3	132
Feeling of discomfort in pit of stomach	40	10	5	86
Hunger pangs	12	1	6	125
Pain in the lower part of the back	26	7	3	104
Pain in the upper part of the back	9	3	2	129
Headache	26	3	1	110
Hearing impairment	8	3	1	132
Asthma eczema false croup	40	8	1	100

Pos and Neg refer to a yes answer and a no answer respectively

Annoyance reactions The questions on annoyance concerned the home and work environments and focused on some ten sources of annoyance in each Among the agents in the home environment can be mentioned traffic noise industrial noise dust and soot and among those in the occupational environment were noise lightning and temperature

The two methods of inquiry showed partly strong discrepancies stemming from the way in which the response alternatives were formulated For each source of annoyance the respondent was instructed to fill in either annoyed or not annoyed or somewhat annoyed   A considerable number of individuals did not respond in either of these two ways since they did not consider them selves as being exposed to the agent in question Such blank responses

Table 8 Alcohol consumption Agreement (in percent of the total number of answers in ther respective consumption groups)

	N	Agreement	Higher value in the interview	Lower value in the interview
Consumption of hard liquor according to the questionnaire				
0 bottles month	48	96	4	0
1	82	59	0	41
2	9	89	0	21
3	4	75	0	25
4 7	6	50	0	50
	149			
Consumption of wine according to the questionnaire				
0 bottles/month	57	81	19	0
1	51	55	12	33
2	24	75	0	25
3	3	67	0	33
4-6	13	92	0	8
7 30	1	100	0	0
	149			
Consumption of beer according to the questionnaire				
0 bottles/month	34	62	38	0
1 5	40	60	7	33
6-10	37	89	8	3
11 25	24	50	0	50
26-	14	29	14	57
	149			

The tendency to state a higher consumption in a mail questionnaire than in a personal interview is familiar from earlier investigations (Björkman 1971) The respondent seems more at ease about revealing his behaviour in an emotionally loaded area on a mail questionnaire

Physical activity Construction of questions on physical activity was based upon the questionnaire used in the diet and exercise survey of National Board of Health and Welfare in Sweden In the present questionnaire the battery of questions was shortened while in the personal interview the original version was employed

The degree of agreement between the questionnaire and interview responses as to physical activity at work has been evaluated according to Table 9

The distribution of responses in the questionnaire on the grades of activity turned out to be 29% 20% 48% and 8% respectively The third catergory was chosen by almost half of the respondents depending on the fact that women not gainfully employed had chosen this alternative It was found that 94% of the

Table 7   Smoking history   agreement in absolute numbers

		Interview Never smoked	Present smoker	Former smoker
Questionnaire	Never smoked	32	0	3
	Present smoker	1	75	2
	Former smoker	0	2	25

Drinking habits   Information on drinking habits has normally been assessed through comprehensive questionnaires   Due to space limitations   the present questionnaire deals with this subject in a short battery of questions   The personal interview   on the other hand   was designed to probe the matter more deeply   The validity of the interview form has been studied earlier (Björkman 1971)

On the basis of the interview questions on frequency and quantity   a measure of consumption corresponding to the questionnaire s   unit of response was calculated i e   number of bottles per month   The degree of agreement between interview and questionnaire with regard to different types of alcohol is shown in Table 8 It is seen that individuals with a   low   consumption of hard liquor   beer and wine   i e   less than one bottle per month   overestimate their consumption by responding   one bottle per month   on the questionnaire   Some respondents however   followed the accepted practice for rounding off numbers giving the value 0 on the questionnaire if they consumed less than 1/2 bottle   Since the goal here with regard to drinking habits is to set up a rough ordinal classification   e g   into low   medium   and high consumers   the impaired precision of the questionnaire should be negligible

Food habits In the questionnaire structured questions were posed regarding a series of various foods The respondent was asked to rate his frequency of consumption on a five-level scale In the personal interview open questions were posed concerning the same foods The interview responses were coded afterwards in terms of the questionnaire categories The agreement between the questionnaire and the interview is illustrated in Table 10

Table 10 Food habits Agreement

	Agreement
Grilled or deep-fried food	97%
Pan-fried or roasted food	96%
Pure meat	98%
Sausage and hot dogs	92%
Liver kidney blood or other organ food	99%
F sh	96%
Shelled seafood	100%
Rice and r ce dishes	91%
Flour based foods (porridge dry cereals pancakes etc)	91%
Egg and egg dishes	95%
Vegetables and root foods	96%
Fruit	95%
Milk sour milk yoghurt or cheese	100%

The agreement varies between 91% (rice dishes flour-based food) and 100% (shelled seafood) The results indicate a high degree of stability in the pattern of response In this evaluation a devation of one unit on the ordinal rating scale has been accepted Moreover the reliability of the responses was checked by comparing the number of meals per day calculable from the frequency with which the respondent had certain main dishes (how much fish etc) versus the number of meals per day stated directly Whenever the two figures differed by less than 0 5 units agreement was considered to exist the resulting agreement was 97% The questionnaire s question on food habits were concluded to serve well the purposes they were meant to serve

Psychosocial status Psychosocial status has been measured in accordance with Eysenck s theory of personality whereby the individual is rated with regard to introversion-extraversion and psychosocial instability respectively A measurement based on the complete Eysenck Personality Inventory would not have been poss ble The number of items comprising the instrument of measurement has been reduced from 24 to 9 per dimension The item selection has been evaluated in a separate study (Floderus 1974)

W th regard to the feasibility of the psychosocal scale in a mail questionnaire the mai concern would be that the respondents would feel less inclined to answer such personal questions causing a large internal non-respons The

respondents gave consistent questionnaire and interview responses

The question regarding leisure time exercise in the questionnaire failed to make distinctions within the large group of individuals with a low level thereof About 80% of the respondents chose either of two responses I do not exercise at all apart from walks now and then and I exercise regularly but lightly through for instance swimming skiing brisk walking or gardening The personal interview employed a seven level subjective rating scale on which the respondents could indicate how much they exercised as well as a detailed question as to type of activity frequency and amount of exertion The frequency and grade of exertion were combined into an index according to which the individuals were classified The subjective ratings showed a strong association with the index for one and the same individual The coefficient of correlation $r_{xy} = 0\ 87$ indicated that a subjective rating aptly describes physical activity The question on the mail questionnaire regarding leisure time exercise has therefore been replaced with this rating scale

Table 9 Comparison between categories on the questionnaire and the interview regarding physical activity at work

Questionnaire	Interview
Mainly sedentary work	Entirely sedentary work (e g office worker ticket-checker student)
	Sedentary shop worker (e g inspector light assembly work driver s job without loading and unloading)
Work which requires quite a bit of standing and walking but which does not demand other physical activity	Work which requires standing and walking and sitting but which does not demand other physical activity (e g sale clerk certan industrial workers dentists)
Work which requires standing and walking but also requires lifting and carrying	Work indoors which requires standing and walking but also calls for lifting and carrying (e g waiter light warehouse work)
	Work outdoors which requires standing and walking but also calls for lifting and carrying (e g work at a filling station driver s job with loading and unloading park work)
Heavy manual labor	Heavy manual labor indoors (e g shop and factory work with heavy tasks)
	Heavy labor outdoors (e g forestry worker farmer road-worker construction worker)

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internal non response for the questions was low however varying between 0 3 and 0 7 throughout Out of the total group investigated 95% were possible to classify with respect to the instability dimension and the corresponding figure for the introversion extraversion dimension amounted to 92% Extremely few subjects had left more than one item of each dimension unanswered

Conclusions

The external non response with which the mail questionnaire was met in the pilot investigation was 18% In view of the questionnaire s size and diffi culty this result can be regarded as very good If the data collection for the twin material meets with a similar non response percentage the number of complete pair-responses could be expected to be 67% Experience from earlier twin investigations leads us to consider this an underestimate

The pilot investigation revealed that only a few of the batteries of questions contained elements which might be difficult for the respondent to answer Accordingly certain revisions e g regarding the annoyance questions have been introduced into the questionnaire proper

DATA COLLECTION

The data collection covered a population of same-sexed twin pairs born between 1926 and 1958 on whom information as to address was available for at least one member of the pair The number of pair which fulfilled these requirements was 21 149 of which 18 309 were pairs for whom the addresses of both twins were known and 2 840 were pairs for whom an address of only one was known

Procedure

A parcel containing an introductory letter a questionnaire and a stamped envelope addressed to the investigators is sent to each twin The twins whose partners addresses are unknown receive in addition a letter requesting that they supply that information After a few days each twin receives a letter reminding him or her to fill in and return the questionnaire Those who still do not respond receive a new parcel about every fourteenth day The contents of these parcels are identical to those of the first one The accompanying letters however are so worded as successively to escalate the pressure on the person The maximum numbe of reminders with full sets of enclosures is 4 meaning that the number of communications sent to those not answering total 6 Those who answer the questionnaire in an incomplete manner are sent a letter requesting further information

Computer support is required to enable such a bulk of addressing and to monitor the in-coming responses For this reason a file containing information on the twins are necessary The name and address of every ndividual are the initial elements in this registry A number of codes have been devised for marking the in coming items one ind cates whether a response has been rece ved another ndicates whether incomplete responses have been completed upon request still anothe indicates responses to the so-called partner letter Every twin is assigned a number which links twin records in the file to the mail questionnaire The twins for whom an address is ava lable on both members of the pa have been assigned numbers from 1 to 36 618 while those for whom the address of only one member is known have been assigned numbers from 50 001 and 55 680

The incom ng responses are handled at a number of checkpoints The first checkpoint includes removing the filled in questionnaires from the envelopes stamping the date on them and checking them off on a special form The in coming mail s also sorted into different categories returned not known at th s address responses containing supplementary information and first time responses The different categories are then subdivided at a secondary check point at which time the items are subjected to another checking off In othe words every ncoming communication is checked off twice the first t me merely to ndicate that it has arrived and the second time in more detail This procedure allows control of the check marks At the secondary check points the responses are examined for completeness the partners letters and other letters are taken care of and supplementary information is inspected and tra sferred onto questionnaire originally filled in The routine of communications is shown in more detail in the organizational scheme *Figure 4*

After computer zed control of the check marks the twin registry is updated ith these and three different lists of address labels are produced one for reminders without a pa tner letter one for reminders with a partner letter and one for the requests fo information which the respondent has failed to

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complete The flow chart in Figure 5 shows the scheme for dispatching the various letters

Actual data collection

The data collection procedure was carried out during the period January March 1973

Parcels returned marked not known at this address and the like numbered 3 685 These were sorted according to county and birth number and listed The lists were sent to the respective county boards with a request for up-to-date addresses Addresses unobtainable in this manner were traced through telephone inquiries at various county boards and parish registrars offices

In 142 cases there was some other kind of mistake in the address These were also traced ty telephone contacts with various county boards

In 420 cases the respondent stated that he or she was not a twin Since such mistakes would stem from faulty identification during the search for the twins addresses a real twin must exist somewhere for every mistaken twin These were traced through contacts with the parish registrars offices and the county boards In a few cases the respondent turned out to be a twin even through he was not aware of it

The responses to the 2 840 partner letters totalled 2 007 In most cases about 1 500 the response was that the partner was dead

After the twin registry had been updated with new addresses etc a second of the data collection procedure was embarked upon during December 1973 and January and February 1974 In principle only the twins who could not be contacted during the first round were approached this time but still another reminder was sent to those who had not responded at all during the first round Some return to sender - unknown at this address responses were also received this second round but these were so few as to be easily handled as soon as they arrived

The coding i e the quantification of response and their conversion to punched cards of the incoming questionnaires was performed during the fall of 1973 and the spring of 1974 A special form for the coding was constructed in order to minimize errors Responses to open questions i e those answerable by a word or a statement were written out and punched as regular text

From the key punched base the material was punched and verified The ca 250 000 punch cards resulting were transferred to a magnetic tape subjected to meticulos computerized checking and edited

ROUTINE FOR RECEPTION OF COMMUNICATIONS

Incoming items in the mail					Incoming telephone calls
The items are opened stamped w th the date and registered on a verification form Sorting takes place according to the categories listed below					Registration with regard to nature of conversation made on a special verification
Postal returns	Incoming responses	Incoming completions	Information on partner	Other	
1 Registration on ve ification form 2 If new address indicated on envelope this is coded onto the punch material base 3 Postal return filed	1 Responses examined as to their completeness 2 If the response is complete registration is made on the verification form and the response is filed 3 If the response is incomplete registration is made on the verification form Request for the information needed is prepared and the questionnaire is filed	1 Registration on verification form 2 Material coded and entered on the original questionnaire 3 Filing	1 Registration on verification form 2 If the partner is dead registration of this is made with a special code 3 Information filed	Information concerning Refusal Illness Away on travel Emigration Not a twin etc 1 Registration on verification form 2 Information filed	

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Results

On several previous occasions it has been mentioned that the twin registry can function both as a registry of individuals and as a registry of twin pairs Accordingly in presenting the results of the data collection procedure these two populations will be treated separately

Population of individuals

From a total population of 42 294 individuals responses have been obtained from 32 374 To judge the quality of the data collection procedure the number of responses should be seen in relation to the number of individuals who could be contacted i e those who actually received the questionnaire The table below shows in addition to the number of responses obtained the numerical values for non-contacted twins according to reason insofar as these could be established via letters and telephone calls or otherwise

Total number of individuals	42 294
Address unknown	1 216
Deceased	1 939
Severely ill	143
Long term stay abroad	37
Emigrated	4
Technical non response total	3 339
Individuals who could be contacted (as far as is known)	38 955
Number of responses	32 374

Technical non response refers to the sum of the individuals who for some known reason could not be contacted Seen then as the number of responses in relation to the number of individuals who could be contacted the response rate is somewhat over 83% Considering the scope of the questionnaire and the resistance felt today toward answering a mail questionnaire this can be described as a good result

Pair pop latio

The table below shows the number of incoming responses

ROUTINE FOR DISPATCHMENT OF VARIOUS COMMUNICATIONS

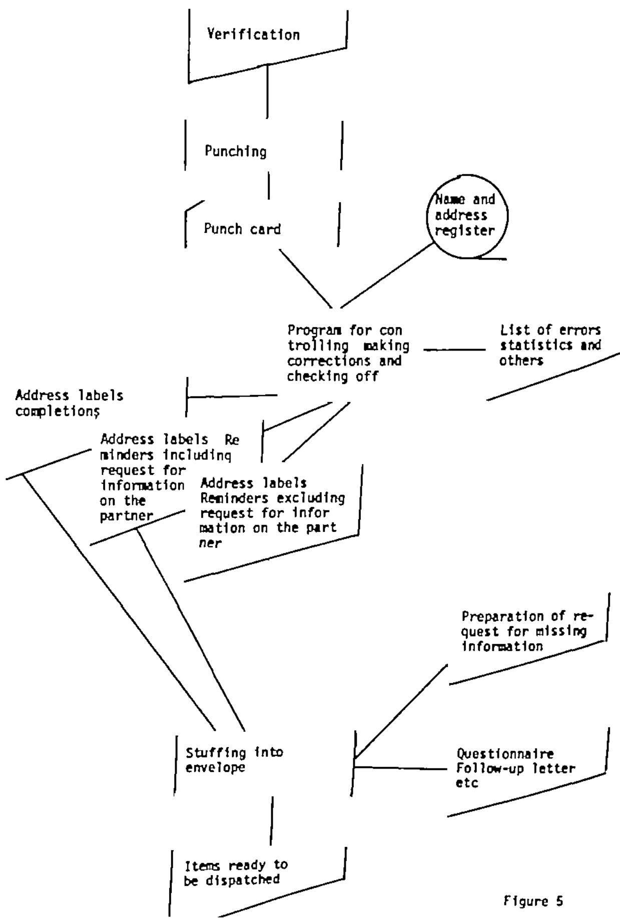


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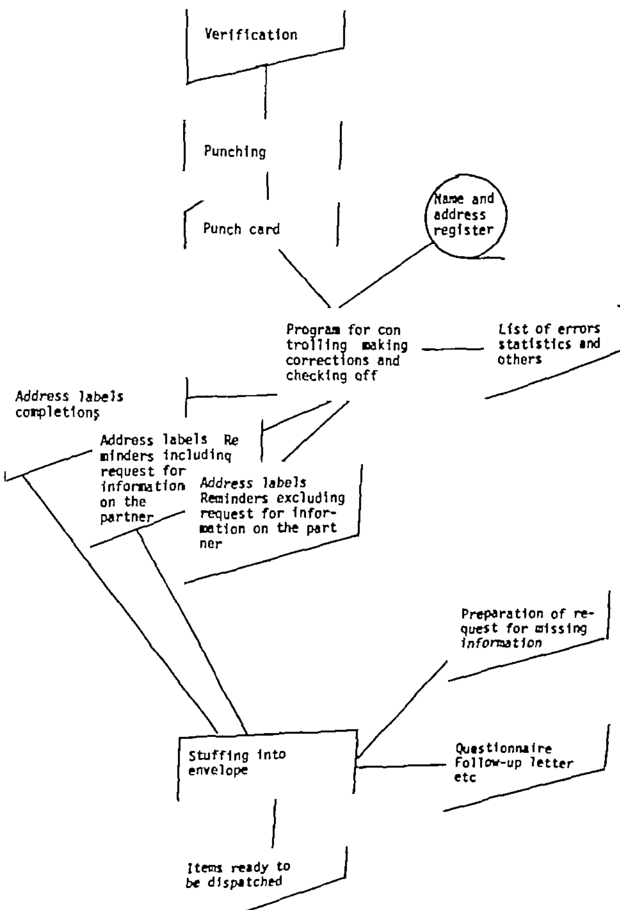
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ROUTINE FOR DISPATCHMENT OF VARIOUS COMMUNICATIONS



DATA CONTENTS

In this section the contents of the twin registry will be presented It is essential to know the limitations inherent in data of this type These limitations must be borne in mind by anyone who is planning a twin study or who wishes to draw conclusions from the present material

Non response is one problem another is that a twin material is not a representive sample of the population in a statistical sense A third problem consists of possible systematic errors due to self selection in variables studied In most cases a twin study is composed of comparisons between groups or between individuals in pairs in which the one twin is more exposed than the other with regard to some variable Such studies resemble experiments with an experiment group and a control group but self selection remains to a certain degree Another problem albeit more trivial arises in estimation situations A statistical dependence exist between individuals in a pair and can not simply be overlooked These problems are not confined to twin investigations Consider for example the practice of using results from experiments on animals to draw conclusions concerning human beings The problems of validity non-response and group comparability are common to most survey nvestigations

The twin registry can be regarded as consisting of 3 different parts the birth registry the address registry and the questionnaire registry

The birth registry includes data from all twin births during 1926-1967 which could be found in birth records and birth notices Among the information stored are

- date of birth and sex of twins born 1926-1949
- national registration number for twins born 1950-1967
- name
- county of b rth municipality parish coded according to the nomenclature of the Central Bureau of Statistics
- born nside or outside a legally registered marriage
- i formation as to whether the twin died in infancy

The address registry covers twin pairs in which neither is marked deceased on the birth record o birth notice Information on birth and as to whether or not the twin could be located is given The following data are entered on the locatable twins

- name address and postal code
- current and previous residence This information includes year residence esta blished county municipality parish taxation district and property notation
- codes more or less complete for legal incompetence marital status income and kinship denotation By kinship denotation is meant the mother s national registration number

The questionnaire registry contains information on same-sexed twins born between 1926 and 1958 Only pairs in which both survived parturition and in which at least one has been located by name and address are stored This registry possesses the same information as the address registry plus a code system revealing whether or not a response has been obtained and if not why These codes also tell

Total number of pairs	21 147
Responses from both members of pair	13 891
Responses from one member of pair	4 592
Responses from neither member of pair	2 664

Again to evaluate the quality of data collection procedure the number of pairs which could be contacted should be taken into account The following table shows the distribution of the known reasons that contact could not be made

Total number of pairs	21 147
Address missing for one or both in pair	1 173
One or both in pair deceased	1 934
One or both in pair severely ill	124
One or both in pair abroad for long term stay	27
One or both in pair emigrated	4
Total	3 262
Concordant with regard to being impossible to contact	107
Number of pairs which could not be contacted	3 155
Number of pairs (as far as is known) which could be contacted	17 992

The proportion of pairs from whom responses from both members were received in relation to the maximum number of contacted is a little more than 77%

Internal non response

The non response can be estimated at about 17% in the individual population and about 23% in the pair population Added to this comes internal non response which refers to unanswered questions The internal non response frequency is difficult to gauge within certain areas of inquiry For example the respondent must be gainfully employed in order to answer the questions on the work situation

Certain questions on tobacco smoking are also difficult to evaluate If for instance the respondent states that he smokes a certain number of filtered cigarettes per day but leaves the question as to how many without filter blank it is most likely that he only smokes filtered cigarettes However it can also be that he has forgotten to answer this question

The internal non response in the majority of the questions which could be thus evaluated was at most 3% The questions on pharmaceuticals had a higher internal non response 4 10%

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whether a request has been sent for information which the twin has failed to fill in and in such cases whether or not the twin has furnished the missing information Whether or not a response of the so called partner letter has arrived is also coded The dates for all of these items are given If twins have responded to the questionnaire the questionnaire data is stored in the registry

All three registries are organized in such a way as to facilitate the analyzis of their contents anew Certain computer soft ware items for instance for the tabulation of the material are available to aid in such new analyses

The information that the registry encompasses should be of interest to researchers outside the Department of Environmental Hygiene as well The only restriction is that the anonymity of the twins must be guaranteed

In the following the content of the various twin registries is presented in the form of tables The rendition of the data divided into one section for each registry is designed primarily to allow estimates directly or indirectly of the volume of information available

The birth registry

The distribution of the total twin population male pairs female pairs and opposite-sexed pairs as to year of birth is shown in Table 11 and as to county of birth in Table 12 These four categories are further broken down into total number of pairs number of pairs in which one twin was a live birth number of pairs in which both were live births and number of pairs in which both were *stillbirths* By *stillbirths* is meant that the death was noted in the record of birth

Table 11 Year of birth and nature of birth

Year of birth	Total: Tot	Total: Neither still born	Total: One still born	Total: Both still born	Male pairs: Tot	Male pairs: Neither still born	Male pairs: One still born	Male pairs: Both still born	Female pairs: Tot	Female pairs: Neither still born	Female pairs: One still born	Female pairs: Both still born	Opposite sexed pairs: Tot	Opposite sexed pairs: Neither still born	Opposite sexed pairs: One still born	Opposite sexed pairs: Both still born
TOT	54890	44636	6689	3565	18065	14182	2392	1491	16940	13907	1933	1100	19885	16547	2364	974
26-27	5 4	5 7	6 4	6 9	5 4	5 0	6 7	6 9	5 2	5 0	5 8	6 6	6 5	5 3	6 4	7 3
28-29	5 2	4 9	6 2	7 2	4 9	4 6	6 0	6 5	5 1	4 8	5 8	7 6	5 5	5 2	6 8	7 7
30 31	4 8	4 5	6 2	6 0	4 7	4 3	6 2	6 9	4 9	4 7	5 8	5 8	4 9	4 6	6 6	6 5
32 33	4 5	4 3	5 2	5 0	4 2	4 1	4 6	4 9	4 6	4 4	5 2	5 5	4 6	4 4	5 7	4 7
34 35	4 2	4 0	5 4	5 6	4 1	3 9	5 0	4 5	4 3	3 9	6 2	5 9	4 3	4 1	5 0	6 8
36-37	4 4	4 2	5 4	6 0	4 3	4 1	5 4	5 1	4 3	4 1	5 2	6 3	4 6	4 3	5 6	7 1
38 39	4 7	4 5	5 8	5 9	4 7	4 3	5 4	6 6	4 8	4 5	6 0	6 1	4 8	4 6	6 0	4 7
40-41	4 7	4 5	5 4	6 0	4 9	4 6	5 9	6 2	4 6	4 4	5 4	6 0	4 7	4 6	4 9	5 7
42-43	5 7	5 8	6 2	6 6	5 6	5 5	5 7	7 0	5 8	5 7	6 8	5 5	5 7	5 6	6 1	7 3
44 45	6 3	6 0	7 0	8 4	6 3	6 0	7 2	8 5	6 5	6 2	7 6	8 5	6 1	5 9	6 4	8 1
46-47	6 1	6 3	5 6	4 9	5 8	6 1	4 8	5 2	6 3	6 6	5 7	4 3	6 2	6 2	6 5	5 3
48-49	5 4	5 7	4 5	3 5	5 5	5 8	4 6	3 9	5 1	5 4	3 9	2 8	5 5	5 8	4	3 7
50-51	4 8	4 9	4 5	3 8	4 9	5 1	4 5	3 8	4 6	4 7	4 2	4 1	4 8	4 9	4 6	3 7
52 53	4 6	4 8	4 1	3 6	4 7	4 9	4 4	3 5	4 4	4 6	3 8	3 0	4 8	4 9	3 9	4 6
54 55	4 4	4 5	4 0	2 9	4 5	4 6	4 1	3 2	4 3	4 4	4 7	2 5	4 4	4 6	3 3	3 0
56-57	4 2	4 4	3 3	2 7	4 3	4 7	3 1	2 6	4 2	4 4	3 3	3 8	4 1	4 3	3 4	1 5
58 59	4 0	4 1	3 9	3 2	4 2	4 1	4 4	4 0	4 0	4 2	3 7	2 2	3 9	4 0	3 5	3 1
60-61	3 8	4 0	3 2	3 1	3 9	4 1	3 5	3 0	3 8	3 9	3 1	3 1	3 8	4 0	3 1	3 3
62 63	4 2	4 6	2 4	2 3	4 3	4 7	2 6	2 7	4 4	4 8	2 3	2 5	3 9	4 3	2 3	1 4
64 65	4 4	4 7	3 1	3 7	4 5	4 9	3 0	3 7	4 5	4 7	3 2	4 2	4 3	4 5	3 2	3 1
66-67	4 0	4 4	2 3	2 5	4 2	4 7	2 7	2 4	4 3	4 7	2 3	3 7	3 6	4 0	1 9	1 3

whether a request has been sent for information which the twin has failed to fill in and in such cases whether or not the twin has furnished the missing information Whether or not a response of the so called partner letter has arrived is also coded The dates for all of these items are given *If twins have responded to the questionnaire the questionnaire data is stored* in the registry

All three registries are organized in such a way as to facilitate the analyzis of their contents anew Certain computer soft ware items for instance for the tabulation of the material are available to aid in such new analyses

The information that the registry encompasses should be of interest to researchers outside the Department of Environmental Hygiene as well The only restriction is that the anonymity of the twins must be guaranteed

In the following the content of the various twin registries is presented in the form of tables The rendition of the data divided into one section for each registry is designed primarily to allow estimates directly or indirectly of the volume of information available

The birth registry

The distribution of the total twin population male pairs female pairs and opposite sexed pairs as to year of birth is shown in Table 11 and as to county of birth in Table 12 These four categories are further broken down into total *number of pairs number of pairs in which one twin was a live birth* number of pairs in which both were live births and number of pairs in which both were stillbirths By stillbirths is meant that the death was noted in the record of birth

The address registry

Tables 13 and 14 show the distribution of the twin pairs according to year and county of birth respectively Four categories are shown total twin population male pairs female pairs and opposite-sexed pairs Each category has been broken down into four groupings: total pairs and pairs in which both one and neither have been located by name and address

Table 15 shows the individual distribution in residence according to county and degree of urbanization The table shows only the individuals located by name and address Age and sex division has been made The degree of urbanization has been ascertained by checking the municipal code which is standardized by the Central Bureau of Statistics A certain series of numbers stands for rural district another for urban district of moderate dimensions and a third for cities "Metropolis has been created out of the city number series by combining Stockholm Gothenburg and Malmö municipalities

Table 16 depicts concordance-discordance with respect to place of residence and its degree of urbanization A division has been made into male pairs female pairs and opposite-sexed pairs These categories have been further separated according to age The degree of urbanization is categorized in the same way as in Table 15 The numbers under place of residence refer to how many twin pairs live in the same parish the same municipality or the same county Only the pairs in which both members have been located are shown

Table 12 County of birth (län)$^{x)}$ and nature of birth

County of birth	Total: Tot	Total: Neither still born	Total: One still born	Total: Both still born	Male pairs: Tot	Male pairs: Neither still born	Male pairs: One still born	Male pairs: Both still born	Female pairs: Tot	Female pairs: Neither still born	Female pairs: One still born	Female pairs: Both still born	Opposite sexed pairs: Tot	Opposite sexed pairs: Neither still born	Opposite sexed pairs: One still-born	Opposite sexed pairs: Both still born
TOT	54890	44636	6689	3565	18065	14182	2392	1491	16940	13907	1933	1100	19885	16547	2364	974
Stockholms län	13 3	13 5	12 4	12 5	13 5	13 8	12 5	12 5	13 3	13 5	12 4	12 5	13 1	13 2	12 3	12 4
Uppsala län	2 1	2 2	2 2	1 7	2 1	2 1	2 2	2 0	2 3	2 3	2 4	1 7	2 0	2 1	1 9	1 3
Södermanlands län	3 0	2 9	3 0	3 4	3 1	3 1	2 6	3 6	2 7	2 6	2 8	2 8	3 2	3 1	3 5	3 7
Östergötlands län	4 8	4 8	4 3	4 9	4 9	5 0	4 1	5 2	4 8	4 8	4 7	4 3	4 6	4 6	4 2	5 3
Jönköpings län	4 2	4 1	4 7	4 5	4 2	4 2	4 3	4 3	4 1	4 0	4 8	4 6	4 3	4 2	4 9	4 5
Kronobergs län	2 3	2 3	2 4	2 2	2 3	2 3	2 3	1 7	2 3	2 3	2 3	2 3	2 5	2 4	2 7	2 9
Kalmar län	3 6	3 5	4 4	3 6	3 4	3 2	4 3	3 5	3 8	3 7	4 7	3 6	3 7	3 6	4 2	3 8
Gotlands län	1 0	1 0	1 3	1 2	1 0	1 0	1 3	5	9	9	9	1 1	1 2	1 1	1 6	2 5
Blekinge län	2 4	2 4	2 5	2 0	2 3	2 3	2 6	2 3	2 3	2 2	2 7	2 0	2 4	2 5	2 1	1 7
Kristianstads län	4 3	4 3	4 3	3 6	4 1	4 2	3 9	3 2	4 3	4 3	3 9	4 4	4 5	4 5	4 9	3 2
Malmöhus län	8 5	8 8	7 8	6 9	8 6	8 7	8 2	7 4	8 7	9 0	7 3	7 4	8 4	8 6	7 9	5 4
Hallands län	2 3	2 3	2 5	2 1	2 3	2 3	2 3	2 1	2 4	2 3	3 2	2 5	2 2	2 3	2 2	1 6
Göteborgs och Bohus	7 4	7 7	5 7	6 2	7 8	8 1	6 8	6 5	7 4	7 9	5 1	5 5	7 0	7 3	5 1	6 6
Älvsborgs län	4 9	4 9	4 9	4 6	5 1	5 1	5 4	4 6	4 9	4 9	5 2	4 9	4 7	4 7	4 3	4 3
Skaraborgs län	3 6	3 6	3 7	4 6	3 6	3 6	3 4	4 2	3 6	3 4	4 0	5 4	3 7	3 7	3 8	4 5
Värmlands län	3 7	3 6	4 1	3 9	3 5	3 3	3 7	4 3	4 0	4 0	4 0	4 0	3 6	3 5	4 5	3 1
Örebro län	3 1	3 1	3 3	3 3	3 1	3 1	2 8	3 6	3 4	3 3	3 9	3 4	3 0	3 0	3 3	2 7
Västmanlands län	2 7	2 7	2 8	2 6	2 6	2 6	2 8	2 5	2 8	2 9	2 5	2 9	2 8	2 7	3 0	2 6
Kopparbergs län	3 3	3 2	3 6	3 9	3 3	3 3	3 3	4 4	3 1	3 0	3 7	3 3	3 4	3 3	3 9	4 0
Gävleborgs län	4 0	3 9	3 8	5 8	4 1	3 8	4 2	5 8	4 0	4 0	3 4	5 3	4 0	3 9	3 7	6 4
Västernorrlands län	4 3	4 3	4 4	4 1	4 2	4 2	4 3	4 1	4 2	4 1	4 2	4 2	4 6	4 7	4 6	4 1
Jämtlands län	2 3	2 2	2 4	2 8	2 3	2 2	2 6	2 7	2 2	2 2	2 5	2 7	2 2	2 2	2 1	3 0
Västerbottens län	4 2	4 2	4 8	4 1	4 2	4 1	5 0	4 0	4 1	4 1	4 4	3 7	4 3	4 2	4 9	4 6
Norrbottens län	4 6	4 5	4 6	5 4	4 5	4 4	4 8	5 0	4 4	4 2	4 7	5 6	4 7	4 7	4 5	5 7

x) The Swedish län is an administrative unit most nearly corresponding to a county and referred to as such in the text In these tables the word län is retained

The address registry

Tables 13 and 14 show the distribution of the twin pairs according to year and county of birth respectively Four categories are shown total twin population male pairs female pairs and opposite-sexed pairs Each category has been broken down into four groupings total pairs and pairs in which both one and neither have been located by name and address

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Table 16 depicts concordance-discordance with respect to place of residence and its degree of urbanization A division has been made into male pairs female pairs and opposite-sexed pairs These categories have been further separated according to age The degree of urbanization is categorized in the same way as in Table 15 The numbers under place of residence refer to how many twin pairs live in the same parish the same municipality or the same county Only the pairs in which both members have been located are shown

Table 13 Year of birth and results of efforts at location

Year of birth	TOT: TOT	TOT: Both loc	TOT: One loc	TOT: Neither loc	Male pairs: TOT	Male pairs: Both loc	Male pairs: One loc	Male pairs: Neither loc	Female pairs: TOT	Female pairs: Both loc	Female pairs: One loc	Female pairs: Neither loc	Opposite-sexed pairs: TOT	Opposite-sexed pairs: Both loc	Opposite-sexed pairs: One loc	Opposite-sexed pairs: Neither loc
TOT	44636	37590	4853	2193	12182	11775	1651	756	13907	11893	1318	696	16547	13922	1884	741
26-27	5 1	4 0	11 5	11 1	5 0	3 7	10 8	13 1	5 0	4 0	11 2	10 1	5 3	4 1	12 4	10 0
28 29	4 9	3 8	11 4	9 5	4 6	3 5	9 8	9 9	4 8	3 9	11 2	8 3	5 2	3 9	12 8	10 3
30-31	4 5	3 7	9 1	8 4	4 3	3 4	8 7	8 6	4 7	3 9	10 0	7 5	4 6	3 7	8 9	9 0
32 33	4 3	3 7	8 1	6 8	4 1	3 5	7 0	7 0	4 4	3 8	8 8	7 0	4 4	3 7	8 5	6 3
34 35	4 0	3 7	5 9	4 0	3 9	3 7	5 8	4 1	3 9	3 7	5 8	3 9	4 1	3 8	6 1	4 0
36 37	4 2	3 9	6 2	4 6	4 1	3 7	6 3	4 1	4 1	3 7	7 0	4 3	4 3	4 1	5 7	5 3
38 39	4 5	4 3	6 5	4 1	4 3	4 1	6 4	4 1	4 5	4 3	6 2	4 3	4 6	4 4	6 8	3 9
40-41	4 5	4 6	5 3	2 4	4 6	4 4	7 1	2 6	4 4	4 5	4 6	1 9	4 6	4 7	4 2	2 6
42 43	5 6	5 8	4 6	2 4	5 5	5 7	5 2	1 9	5 7	5 9	5 7	2 6	5 6	5 7	6 0	2 7
44 45	6 0	6 2	5 3	3 8	6 0	6 2	5 0	3 4	6 2	6 4	6 0	3 7	5 9	6 2	5 1	4 2
46 47	6 3	6 1	6 4	8 0	6 1	6 0	6 9	5 4	6 6	6 4	6 4	9 3	6 2	6 0	5 9	9 3
48 49	5 7	5 6	4 8	9 0	5 8	5 7	5 1	9 1	5 4	5 4	4 1	7 5	5 8	5 6	5 0	10 4
50-51	4 9	5 2	2 9	3 6	5 1	5 5	2 9	3 4	4 7	4 9	2 7	4 7	4 9	5 3	3 1	2 7
52 53	4 8	5 3	2 3	2 3	4 9	5 4	2 8	2 5	4 6	5 0	2 0	2 7	4 9	5 4	2 0	1 8
54 55	4 5	5 1	1 5	2 6	4 6	5 2	1 5	2 2	4 4	4 8	1 2	3 3	4 6	5 2	1 6	2 4
56 57	4 4	4 7	2 4	4 1	4 7	4 9	3 0	4 4	4 4	4 5	2 8	4 6	4 3	4 7	1 6	3 2
58 59	4 1	4 6	8	1 8	4 1	4 7	9	1 9	4 2	4 7	7	1 4	4 0	4 5	9	2 2
60-61	4 0	4 5	5	2 2	4 1	4 7	8	2 0	3 9	4 4	3	2 6	4 0	4 5	4	2 2
62 63	4 6	5 0	1 4	4 4	4 7	5 2	1 6	5 0	4 8	5 3	1 0	4 0	4 3	4 7	1 5	4 2
64 65	4 7	5 4	7	2 1	4 9	5 6	9	2 5	4 7	5 3	1 0	2 0	4 5	5 2	4	1 6
66-67	4 4	4 9	1 1	2 8	4 7	5 3	1 2	2 6	4 7	5 1	1 1	4 2	4 0	4 5	1 0	1 8

Table 14 County of birth (län)[x] and results of efforts at location

County of birth	TOT: TOT	TOT: Both loc	TOT: One loc	TOT: Neither loc	Male pairs: TOT	Male pairs: Both loc	Male pairs: One loc	Male pairs: Neither loc	Female pairs: TOT	Female pairs: Both loc	Female pairs: One loc	Female pairs: Neither loc	Opposite-sexed pairs: TOT	Opposite-sexed pairs: Both loc	Opposite-sexed pairs: One loc	Opposite-sexed pairs: Neither loc
TOT	44636	37590	4853	2193	14182	11775	1651	756	13907	11893	1318	696	16547	13922	1884	741
Stockholms län	13 5	14 0	10 1	13 0	13 8	14 4	10 6	11 5	13 5	14 0	9 1	13 2	13 2	13 6	10 5	14 4
Uppsala län	2 2	2 2	2 0	1 8	2 1	2 2	1 6	1 6	2 3	2 4	2 0	2 0	2 1	2 0	2 4	1 8
Södermanlands län	2 9	3 0	2 5	3 0	3 1	3 1	2 9	3 6	2 6	2 7	2 3	2 2	3 1	3 2	2 3	3 1
Östergötlands län	4 6	4 8	4 7	5 1	5 0	6 0	4 5	6 1	4 8	4 8	5 0	4 3	4 6	4 6	4 6	4 7
Jönköpings län	4 1	4 2	4 0	3 7	4 2	4 2	3 8	4 2	4 0	4 1	3 6	3 6	4 2	4 2	4 4	3 4
Kronobergs län	2 3	2 4	2 1	2 3	2 3	2 4	1 8	2 9	2 3	2 3	1 7	2 0	2 4	2 4	2 6	2 0
Kalmar län	3 5	3 4	3 8	4 0	3 2	3 1	4 0	3 2	3 7	3 7	3 3	3 4	3 6	3 4	3 8	5 4
Gotlands län	1 0	1 0	1 1	8	1 0	9	1 0	1 6	9	1 0	6	6	1 1	1 0	1 6	1
Blekinge län	2 4	2 3	2 4	2 6	2 3	2 3	2 4	2 4	2 2	2 2	2 6	2 4	2 5	2 5	2 4	3 0
Kristianstads län	4 3	4 2	5 2	5 0	4 2	4 1	4 7	4 6	4 3	4 3	5 2	3 7	4 5	4 2	5 6	6 6
Malmöhus län	8 8	8 6	9 7	9 1	8 7	8 5	10 6	9 0	9 0	8 9	9 0	10 3	8 6	8 5	9 5	8 1
Hallands län	2 3	2 3	2 3	1 8	2 3	2 3	2 1	2 4	2 3	2 3	2 6	0	2 3	2 3	2 4	1 1
Göteborgs och Bohus	7 7	7 9	7 2	7 0	8 1	8 2	7 9	6 9	7 9	8 0	7 7	7 2	7 3	7 5	6 3	7 0
Älvsborgs län	4 9	5 0	4 4	4 4	5 1	5 2	4 3	5 2	4 9	4 9	4 5	4 3	4 7	4 8	4 5	3 6
Skaraborgs län	3 6	3 6	4 0	3 4	3 6	3 5	4 2	4 0	3 4	3 4	3 9	3 4	3 7	3 7	3 9	2 7
Värmlands län	3 6	3 6	3 6	3 4	3 3	3 4	3 1	3 0	4 0	3 9	4 4	4 5	3 5	3 6	3 5	2 8
Örebro län	3 7	3 2	2 4	2 6	3 1	3 2	2 4	2 2	3 3	3 3	2 7	3 9	3 0	3 1	2 2	1 8
Västmanlands län	2 7	2 8	2 2	2 3	2 6	2 7	1 9	2 4	2 9	2 8	3 3	2 3	2 7	2 9	1 8	2 3
Kopparbergs län	3 2	3 3	2 8	3 1	3 3	3 3	3 3	3 0	3 0	3 1	2 0	3 0	3 3	3 4	3 0	3 1
Gävleborgs län	3 9	3 9	4 1	3 9	3 8	3 8	4 1	3 7	4 0	4 0	4 6	4 3	3 9	4 0	3 8	3 6
Västernorrlands län	4 3	4 2	5 2	4 7	4 2	4 1	5 1	3 7	4 1	4 0	5 6	4 3	4 7	4 5	5 1	6 1
Jämtlands län	2 2	2 1	2 8	2 1	2 2	2 1	3 0	1 5	2 2	2 1	3 1	2 2	2 2	2 2	2 3	2 8
Västerbottens län	4 2	4 0	5 6	4 2	4 1	4 0	5 5	4 1	4 1	4 0	6 5	4 6	4 2	3 9	5 9	4 0
Norrbottens län	4 5	4 2	5 7	6 6	4 4	4 1	5 5	7 3	4 2	4 0	5 7	6 2	4 7	4 5	5 8	6 3

x) The Swedish län is an administrative unit most nearly corresponding to a county and referred to as such in the text: In these tables the word län is retained

Table 13 Year of birth and results of efforts at location

Year of birth	TOT				Male pairs				Female pairs				Opposite-sexed pairs			
	TOT	Both loc	One loc	Neither loc	TOT	Both loc	One loc	Neither loc	TOT	Both loc	One loc	Neither loc	TOT	Both loc	One loc	Neither loc
TOT	44636	37590	4853	2193	12182	11775	1651	756	13907	11893	1318	696	16547	13922	1884	741
26-27	5 1	4 0	11 5	11 1	5 0	3 7	10 8	13 1	5 0	4 0	11 2	10 1	5 3	4 1	12 4	10 0
28 29	4 9	3 8	11 4	9 5	4 6	3 5	9 8	9 9	4 8	3 9	11 2	8 3	5 2	3 9	12 8	10 3
30 31	4 5	3 7	9 1	8 4	4 3	3 4	8 7	8 6	4 7	3 9	10 0	7 5	4 6	3 7	8 9	9 0
32 33	4 3	3 7	8 1	6 8	4 1	3 5	7 0	7 0	4 4	3 8	8 8	7 0	4 4	3 7	8 5	6 3
34 35	4 0	3 7	5 9	4 0	3 9	3 7	5 8	4 1	3 9	3 7	5 8	3 9	4 1	3 8	6 1	4 0
36 37	4 2	3 9	6 2	4 6	4 1	3 7	6 3	4 1	4 1	3 7	7 0	4 3	4 3	4 1	5 7	5 3
38 39	4 5	4 3	6 5	4 1	4 3	4 1	6 4	4 1	4 5	4 3	6 2	4 3	4 6	4 4	6 8	3 9
40-41	4 5	4 6	5 3	2 4	4 6	4 4	7 1	2 6	4 4	4 5	4 6	1 9	4 6	4 7	4 2	2 6
42 43	5 6	5 8	4 6	2 4	5 5	5 7	5 2	1 9	5 7	5 9	5 7	2 6	5 6	5 7	6 0	2 7
44 45	6 0	6 2	5 3	3 8	6 0	6 2	5 0	3 4	6 2	6 4	6 0	3 7	5 9	6 2	5 1	4 2
46-47	6 3	6 1	6 4	8 0	6 1	6 0	6 9	5 4	6 6	6 4	6 4	9 3	6 2	6 0	5 9	9 3
48 49	5 7	5 6	4 8	9 0	5 8	5 7	5 1	9 1	5 4	5 4	4 1	7 5	5 8	5 6	5 0	10 4
50 51	4 9	5 2	2 9	3 6	5 1	5 5	2 9	3 4	4 7	4 9	2 7	4 7	4 9	5 3	3 1	2 7
52 53	4 8	5 3	2 3	2 3	4 9	5 4	2 8	2 5	4 6	5 0	2 0	2 7	4 9	5 4	2 0	1 8
54 55	4 5	5 1	1 5	2 6	4 6	5 2	1 5	2 2	4 4	4 8	1 2	3 3	4 6	5 2	1 6	2 4
56-57	4 4	4 7	2 4	4 1	4 7	4 9	3 0	4 4	4 4	4 5	2 8	4 6	4 3	4 7	1 6	3 2
58-59	4 1	4 6	8	1 8	4 1	4 7	9	1 9	4 2	4 7	7	1 4	4 0	4 5	9	2 2
60-61	4 0	4 5	5	2 2	4 1	4 7	8	2 0	3 9	4 4	3	2 6	4 0	4 5	4	2 2
62 63	4 6	5 0	1 4	4 4	4 7	5 2	1 6	5 0	4 8	5 3	1 0	4 0	4 3	4 7	1 5	4 2
64 65	4 7	5 4	7	2 1	4 9	5 6	9	2 5	4 7	5 3	1 0	2 0	4 5	5 2	4	1 6
66-67	4 4	4 9	1 1	2 8	4 7	5 3	1 2	2 6	4 7	5 1	1 1	4 2	4 0	4 5	1 0	1 8

Table 16 Concordance and discordance with respect to place of residence and its degree of urbanization

	TOT				Male pairs				Female pairs				Opposite sexed pairs			
	Tot	Male pairs	Female pairs	Opposite sexed pairs	26-35	36-45	46-58	59-67	26-35	36-45	46-58	59 67	26 35	36-45	46 58	59-67
Place of residence																
Total	37590	11775	11893	13922	2086	2847	4131	2711	2298	2963	3984	2648	2683	3484	4821	2934
Same parish	56 7	64 3	54 7	52 0	27 0	31 5	82 9	98 9	18 6	21 4	70 4	99 4	15 7	16 4	69 5	98 9
Same municipality differ parish	9 2	8 1	9 2	10 0	14 8	15 9	4 6	1	16 4	14 1	7 5	1	16 3	16 2	7 9	3
Same county differ municipality	13 6	11 4	14 3	14 9	21 9	21 9	5 9	5	23 8	23 7	10 9	3	24 3	25 9	10 5	4
Different counties	20 6	16 3	21 9	23 1	36 4	30 7	6 6	4	41 1	40 8	11 1	2	43 8	41 4	12 1	4
Degree of urbanization																
Total	37590	11775	11893	13922	2086	2847	4131	2711	2298	2963	3984	2648	2683	3484	4821	2934
Ru al/Rural	14 1	15 8	12 9	13 7	10 0	9 9	17 9	23 1	7 7	7 2	15 1	20 8	7 5	7 6	16 2	22 4
Rural/Small city	1 6	1 5	1 7	1 7	2 9	3 1	6	1	3 1	3 1	9		3 0	3 2	1 0	
Rural/City	7 4	5 8	7 4	8 7	12 9	10 8	2 5		12 7	14 0	4 3	2	14 9	15 4	5 5	3
Rural/Metropolis	2 8	2 5	3 0	2 8	4 8	4 8	1 3	1	4 4	6 0	1 8		4 5	5 8	1 5	
Small city/Small city	7 2	8 1	6 7	6 9	5 3	5 0	9 9	10 9	3 7	3 7	8 2	10 6	6 1	3 8	8 0	10 6
Small city/City	4 4	3 6	4 9	4 8	7 9	6 5	1 6	1	8 8	8 2	3 5	1	8 8	8 4	2 7	1
Small city/Metropolis	1 0	7	1 2	1 2	1 3	1 3	3		2 7	1 9	7		2 4	1 6	8	1
City/City	44 7	45 9	44 6	44 0	39 2	40 7	48 9	51 6	38 6	37 9	46 9	53 6	36 9	37 1	47 5	53 0
City/Metropolis	5 0	4 2	5 3	5 4	8 1	8 1	2 1	2	9 7	9 4	3 4		10 0	9 5	3 1	1
Metropolis/Metropolis	11 7	12 1	12 2	10 8	7 5	9 7	14 9	13 8	8 7	8 6	15 3	14 7	6 9	7 7	13 7	13 4

Table 15 County of residence the degree of urbanization of place of residence

	+ + T O T + +			+ + + MEN + + +				+ + + WOMEN + + +			
	TOT	Men	Women	26 35	36 45	46 58	59 67	26 35	36 45	46 58	59 67
County of residence[x)]	80039										
TOT	100	40010	40029	7960	5919	13660	8471	8411	10080	13223	8315
Stockholms län	17 4	17 1	17 7	15 3	18 6	16 5	18 1	15 7	20 1	17 4	17 3
Uppsala län	2 6	2 5	2 7	2 4	2 5	2 4	2 5	2 4	2 7	2 8	3 1
Södermanlands län	3 0	3 1	2 9	3 4	3 1	2 9	3 4	2 7	2 8	3 1	3 0
Östergötlands län	4 7	4 7	4 7	5 0	5 0	4 6	4 4	5 0	4 7	4 7	4 4
Jönköpings län	4 1	4 2	4 0	4 4	3 7	4 2	4 4	4 3	3 9	4 1	3 8
Kronobergs län	2 2	2 2	2 2	2 5	2 1	2 1	2 3	2 6	2 0	2 1	2 4
Kalmar län	2 9	2 9	3 0	3 0	2 7	3 0	2 7	3 2	2 8	3 0	3 2
Gotlands län	0 8	8	7	7	8	8	8	6	7	8	7
Blekinge län	2 1	2 2	2 1	2 3	2 3	2 2	2 1	2 2	2 2	1 8	2 2
Kristianstads län	3 7	3 6	3 7	4 0	3 5	3 7	3 3	4 2	3 6	3 7	3 5
Malmöhus län	9 4	9 2	9 6	9 9	9 3	8 8	8 9	10 1	10 1	9 2	9 4
Hallands län	2 4	2 4	2 4	2 5	2 4	2 3	2 5	2 5	2 1	2 4	2 6
Göteborgs och Bohus	8 7	8 6	8 8	8 2	9 4	8 3	8 6	9 0	8 5	8 7	8 9
Älvsborgs län	5 0	5 1	4 8	5 4	4 8	5 4	4 9	4 7	4 8	4 6	5 2
Skaraborgs län	3 3	3 3	3 2	3 2	3 2	3 4	3 5	3 3	2 8	3 3	3 4
Värmlands län	3 2	3 1	3 4	3 7	2 7	3 1	2 8	3 3	3 2	3 4	3 5
Örebro län	3 2	3 1	3 3	3 0	2 9	3 3	3 4	3 3	3 3	3 6	2 9
Västmanlands län	3 1	3 1	3 0	2 8	3 2	3 1	3 4	2 8	3 0	3 2	2 9
Kopparbergs län	3 0	3 2	2 9	3 0	3 1	3 4	3 3	2 9	2 7	3 0	2 9
Gävleborgs län	3 5	3 6	3 5	3 6	3 8	3 6	3 4	3 8	3 3	3 5	3 5
Västernorrlands län	3 5	3 6	3 4	3 7	2 9	4 1	3 3	3 6	3 5	3 5	3 2
Jämtlands län	1 6	1 6	1 5	1 6	1 6	1 7	1 4	1 6	1 2	1 6	1 7
Västerbottens län	3 2	3 3	3 1	2 9	3 3	3 5	3 2	3 2	3 1	3 3	2 9
Norrbottens län	3 4	3 5	3 3	3 6	3 1	3 7	3 5	3 2	3 0	3 5	3 5
DEGREE OF URBANIZATION OF PLACE OF RESIDENCE											
Rural	20 0	20 7	19 2	20 1	19 7	20 5	23 1	18 1	18 9	18 8	21 5
Small	10 8	11 0	10 6	11 6	10 6	10 9	10 9	11 2	10 2	10 4	10 /
City	53 1	52 7	53 5	53 5	53 3	52 1	52 2	53 7	53 9	53 0	53 5
Metropolis	16 1	15 6	16 7	14 9	16 4	16 5	13 8	16 9	17 1	17 7	14 2

x) The Swedish lan is an administrative unit most nearly corresponding to a county and referred to as such in the text In these tables the word län is retained

Table 17 County of residence; the degree of u banization of place of r sid nce

	T O T			MEN			WOMEN		
	TOT	MEN	WOMEN	26 35	36 45	46 58	26 35	36 45	46 58
County of residence[x)]									
TOT	39456	19698	19758	4805	6114	8571	5113	6157	8137
Stockholms län	17 5	16 9	18 1	15 6	18 2	16 5	16 3	20 0	17 5
Uppsala län	2 6	2 5	2 7	2 3	2 4	2 7	2 2	2 6	3 0
Södermanlands län	3 0	3 0	2 9	3 5	3 3	2 5	2 8	2 9	3 0
Östergötlands län	4 8	4 9	4 8	5 3	5 1	4 6	4 8	4 6	4 8
Jönköpings län	4 0	4 0	4 1	4 2	3 8	4 1	4 2	4 0	4 1
Kronobergs län	2 1	2 2	2 1	2 4	2 1	2 1	2 6	1 9	2 0
Kalmar län	3 0	2 9	3 1	2 7	2 8	3 0	3 4	3 0	2 9
Gotlands län	7	7	7	6	9	7	6	8	8
Blekinge län	2 1	2 2	2 0	2 2	2 1	2 3	2 2	2 1	1 7
Kristianstads län	3 7	3 7	3 7	4 1	3 5	3 7	4 0	3 5	3 8
Malmöhus län	9 5	9 2	9 8	9 2	9 2	9 2	9 6	10 5	9 4
Hallands län	2 3	2 3	2 3	2 7	2 2	2 2	2 5	1 9	2 3
Göteborgs och Bohus	9 0	9 1	8 9	8 9	9 9	8 6	9 3	8 4	9 0
Älvsborgs län	6 0	5 4	4 6	5 7	5 1	5 4	4 9	4 9	4 3
Skaraborgs län	3 1	3 2	3 0	3 3	3 2	3 2	3 4	2 6	3 1
Värmlands län	3 2	3 0	3 4	3 7	2 4	3 0	3 4	3 1	3 6
Örebro län	3 3	3 0	3 6	2 8	2 7	3 3	3 3	3 4	3 9
Västmanlands län	2 9	2 9	3 0	2 7	3 0	2 9	2 9	3 1	9
Kopparbergs län	2 9	3 1	2 7	2 8	3 0	3 3	2 7	2 6	2 9
Gävleborgs län	3 6	3 7	3 5	3 7	3 9	3 5	3 8	3 2	3 5
Västernorrlands län	3 6	3 7	3 4	3 5	3 1	4 4	3 3	3 6	3 3
Jämtlands län	1 5	1 6	1 4	1 6	1 7	1 6	1 6	1 4	1 5
Västerbottens län	3 3	3 3	3 2	3 0	3 3	3 6	3 3	3 1	3 3
Norrbottens län	3 2	3 4	3 1	3 4	3 1	3 6	3 2	2 7	3 4
Degree of urbanization									
TOT	39456	19698	19758	4805	6114	8571	5113	6157	8137
Rural	19 2	19 9	18 5	20 1	19 4	20 1	18 5	18 7	18 7
Small city	10 8	11 0	10 7	11 2	10 6	11 2	11 0	10 4	10 7
City	52 9	52 7	53 1	53 5	53 2	52 0	53 4	53 8	52 4
Metropolis	17 1	16 5	17 7	15 2	16 8	16 8	17 1	17 1	18 2

x) The Swedish län is an administrative unit most nearly corresponding to a county and referred to as such in the text In this table the word län is retained

The questionnaire registry

The data in the questionnaire registry are tabulated in two sections The first section (Tables 17 21) presents the administrative data The second section (Tables 1 13 for A and B series) deals with data from the question naire

Administrative data

Table 17 shows the residential distribution of the individuals as to county and degree of urbanization Urbanization is defined in the same manner as in the address registry The material is separated into sex and year of birth The entire population is embraced apart from the 2 840 twins for whom this infor mation is lacking

Table 18 is identical with Table 17 but the table is comprised of only those persons who responded to the questionnaire

Table 19 divides the twins according to year of birth with subdivisions for total population pairs in which both members filled in the questionnaire pairs in which one member did so and pairs in which neither did so The material is also presented with a division according to sex

In Table 20 concordance/discordance is illustrated with respect to place of residence and its degree of urbanization The numbers represent twin pairs divided as to sex and age The table shows the total number of pairs as well as the pairs in which both members filled in the questionnaire Urbanization has been defined in the same way as in the address registry

Table 21 divides the material in the same way as Table 20 Here pairs in which both members have filled in the questionnaire are presented in terms of zygosity sex and age

Table 17 County of es de ce the degree of urbanization of place of reside e

	T O T			MEN			WOMEN		
	TOT	MEN	WOMEN	26 35	36 45	46 58	26 35	36 45	46 58
County of residence[x)]									
TOT	39456	19698	19758	4805	6114	8571	5113	6157	8137
Stockholms län	17 5	16 9	18 1	15 6	18 2	16 5	16 3	20 0	17 5
Uppsala län	2 6	2 5	2 7	2 3	2 4	2 7	2	2 6	3 0
Södermanlands län	3 0	3 0	2 9	3 5	3 3	2 5	2 8	2 9	3 0
Östergötlands län	4 8	4 9	4 8	5 3	5 1	4 6	4 8	4 6	4 8
Jönköpings län	4 0	4 0	4 1	4 2	3 8	4 1	4 2	4 0	4 1
Kronobergs län	2 1	2 2	2 1	2 4	2 1	2 1	2 6	1 9	2 0
Kalmar län	3 0	2 9	3 1	2 7	2 8	3 0	3 4	3 0	2 9
Gotlands län	7	7	7	6	9	7	6	8	8
Blekinge län	2 1	2 2	2 0	2 2	2 1	2 3	2 2	2 1	1 7
Kristianstads län	3 7	3 7	3 7	4 1	3 5	3 7	4 0	3 5	3 8
Malmöhus län	9 5	9 2	9 8	9 2	9 2	9 2	9 6	10 5	9 4
Hallands län	2 3	2 3	2 3	2 7	2 2	2 2	2 5	1 9	2 3
Göteborgs och Bohus	9 0	9 1	8 9	8 9	9 9	8 6	9 3	8 4	9 0
Älvsborgs län	5 0	5 4	4 6	5 7	5 1	5 4	4 9	4 9	4 3
Skaraborgs län	3 1	3 2	3 0	3 3	3 2	3 2	3 4	2 6	3 1
Värmlands län	3 2	3 0	3 4	3 7	2 4	3 0	3 4	3 1	3 6
Örebro län	3 3	3 0	3 6	2 8	2 7	3 1	3 3	3 4	3 9
Västmanlands län	2 9	2 9	3 0	2 7	3 0	2 9	2 9	3 1	2 9
Kopparbergs län	2 9	3 1	2 7	2 8	3 0	3 3	2 7	2 6	2 9
Gävleborgs län	3 6	3 7	3 5	3 7	3 9	3 5	3 8	3 2	3 5
Västernorrlands län	3 6	3 7	3 4	3 5	3 1	4 4	3 3	3 6	3 3
Jämtlands län	1 5	1 6	1 4	1 5	1 7	1 6	1 5	1 4	1 5
Västerbottens län	3 3	3 3	3 2	3 0	3 3	3 6	3 3	3 1	3 3
Norrbottens län	3 2	3 4	3 1	3 4	3 1	3 6	3 2	2 7	3 4
Degree of urbanization									
TOT	39456	19698	19758	4805	6114	8571	5113	6157	8137
Rural	19 2	19 9	18 5	20 1	19 4	20 1	18 5	18 7	18 7
Small city	10 8	11 0	10 7	11 2	10 6	11 2	11 0	10 4	10 7
City	52 9	52 7	53 1	53 5	53 2	52 0	53 4	53 8	52 4
Metropolis	17 1	16 6	17 7	15 2	16 8	16 8	17 1	17 1	18 2

x) The Swedish län is an administrative unit most nearly corresponding to a county and referred to as such in the text In this table the word län is retained

Table 18 County of residence the degree of urbanization of place of residence

	+ + T O T + +			+ + + MEN + + +			+ + + WOMEN + + +		
County of residence[x)]	TOT	MEN	WOMEN	26 35	36 45	46 58	26-35	36 45	46 58
TOT	31805	15336	16469	3773	4828	6687	4274	5239	6822
Stockholms län	17 3	16 6	17 9	15 3	18 1	16 2	15 8	19 8	17 6
Uppsala län	2 7	2 6	2 8	2 4	2 5	? 8	2 3	2 7	3 1
Södermanlands län	3 0	3 1	3 0	3 5	3 3	2 7	2 8	3 7	3 1
Östergötlands län	5 1	5 3	4 9	5 8	5 4	4 9	4 9	4 8	5 0
Jönköpings län	4 0	4 0	4 0	4 2	3 7	4 ∠	4 2	3 7	4 2
Kronobergs län	2 3	2 3	2 2	2 6	2 3	2 2	2 7	1 8	2 2
Kalmar län	3 0	3 0	3 1	2 7	3 0	3 1	3 2	2 9	3 1
Gotlands län	8	8	8	7	9	7	6	8	9
Blekinge län	2 0	2 1	2 0	1 8	2 1	2 3	2 3	2 1	1 7
Kristianstads län	3 6	3 6	3 6	4 3	3 4	3 4	3 7	3 3	3 8
Malmöhus län	9 2	8 8	9 6	8 8	8 7	8 9	9 5	10 3	9 2
Hallands län	2 2	2 3	2 1	2 7	2 3	2 0	2 4	1 7	2 3
Göteborgs och Bohus	8 8	9 0	8 6	9 1	9 8	8 3	9 1	8 4	8 5
Älvsborgs län	4 9	5 4	4 5	5 6	5 1	5 4	5 1	4 9	3 9
Skaraborgs län	3 1	3 4	2 9	3 4	3 4	3 3	3 5	2 3	2 9
Värmlands län	3 1	2 9	3 4	3 2	2 3	3 2	3 4	3 4	3 3
Örebro län	3 3	2 9	3 6	2 7	2 5	3 3	3 4	3 4	3 9
Västmanlands län	3 0	2 9	3 1	2 8	3 1	2 8	3 0	3 3	3 0
Kopparbergs län	3 0	3 1	2 8	3 0	2 9	3 4	2 8	2 7	3 0
Gävleborgs län	3 7	3 8	3 6	3 9	4 2	3 4	3 9	3 3	3 6
Västernorrlands län	3 6	3 6	3 5	3 4	3 0	4 3	3 3	3 7	3 5
Jämtlands län	1 5	1 6	1 4	1 6	1 5	1 7	1 4	1 4	1 5
Västerbottens län	3 5	3 6	3 4	3 2	3 4	3 9	3 5	3 2	3 4
Norrbottens län	3 2	3 4	3 1	3 3	3 1	3 7	3 3	2 7	3 4
Degree of urbanization									
TOT	31805	15336	16469	3773	4828	6687	4274	5239	6822
Rural	19 7	20 4	19 1	21 0	19 8	20 5	19 0	19 5	19 1
Small city	11 0	11 1	10 9	11 3	10 7	11 4	11 3	10 5	10 9
City	53 2	53 1	53 2	53 5	53 8	52 5	53 5	53 5	52 5
Metropolis	16 1	15 3	16 8	14 2	15 8	15 6	16 2	16 5	17 5

x) The Swedish lan is an administrative unit most nearly corresponding to a county and ref rred to as such in the text In this table the word län is etained

T ble 19 Yea of bi th and response or non-response

	+ T O T				+ MEN + + +				+ + + WOMEN + + +			
	TOT	Both re-sponded	One re sponded	Neither re-sponded	TOT	Both re sponded	One re-sponded	Neither re sponded	TOT	Both re-sponded	One re-sponded	Neither re sponded
Year of birth												
TOT	20910	13664	4511	2735	10553	6417	2541	1595	10357	7247	1970	1140
26-27	58	47	85	64	57	44	85	62	58	50	85	67
28-29	55	47	79	55	53	46	75	50	56	48	84	62
30-31	55	49	74	55	51	46	66	50	59	52	85	62
32 33	51	45	70	51	49	42	67	48	53	48	73	55
34 35	49	47	61	39	49	47	61	39	49	47	59	38
36 37	50	48	63	40	50	49	59	42	50	48	68	38
38 39	56	53	66	51	55	52	64	53	56	55	68	46
40-41	60	58	69	54	61	57	72	61	59	59	66	45
42-43	71	73	73	56	71	74	76	52	71	73	70	62
44 45	77	80	71	72	76	77	76	71	78	82	65	73
46-47	77	75	83	80	76	72	79	83	79	78	87	75
48-49	69	72	62	67	72	76	64	69	66	69	59	63
50-51	63	66	48	69	65	71	48	69	60	63	47	69
52 53	62	67	44	62	64	70	49	63	59	65	37	61
54 55	58	60	20	111	61	64	22	108	56	57	18	115
56-57	57	72	22	4	59	75	23	52	55	69	20	29
58	28	37	6	22	27	38	7	19	29	36	5	27

Table 20 Concordance/discordance with regard to place of residence degree of urbanization All twin pairs

–

	Total				Men			Women	
	Tot	Men	Women	26 35	36 45	46 58	26 35	36- 45	46 58
Place of residence									
All	18309	9064	9245	2063	2817	4105	2252	2896	3947
Same parish	47 8	53 9	41 8	27 0	31 7	83 3	18 9	21 7	70 8
Same municipality differ parish	11 2	10 5	11 8	14 9	16 0	4 5	16 5	14 3	7 5
Same county differ municipality	16 4	14 6	18 2	21 7	22 1	5 8	24 2	23 7	10 7
Different counties	24 6	21 0	28 1	36 4	30 2	6 3	40 5	40 3	11 0
Degree of urbanization									
All	18309	9064	9245	2063	2817	4105	2252	2896	3947
Rural/Rural	3	5	2	2	3	7	1	2	3
Rural/Small city	1 0	9	1 1	1 9	1 2	2	1 6	1 6	5
Rural/City	4 0	3 7	4 2	6 3	5 3	1 2	6 0	6 1	1 9
Rural/Metropolis	1 9	1 7	2 0	2 7	2 7	6	2 4	3 1	1 0
Small city/Small city	6 4	7 3	5 6	5 3	5 0	9 9	3 7	3 8	8 2
Small city/City	5 5	4 6	6 3	7 8	6 6	1 6	9 0	8 1	3 4
Small city/Metropolis	1 2	9	1 6	1 3	1 2	3	2 7	1 9	7
City/City	43 0	44 1	41 9	39 2	40 9	49 1	38 5	38 1	47 0
City/Metropolis	6 1	5 4	6 9	8 2	8 0	1 9	9 5	9 3	3 3
Metropolis/Metropolis	30 5	30 9	30 1	27 0	28 8	34 5	26 5	27 9	33 7

Table 20 (cont d) Responses from both members of the pair

	Total			Men			Women		
	Tot	Men	Women	26 35	36 45	46 58	26 35	36 45	46 58
Place of residence									
All	13663	6436	7227	1415	1962	3041	1721	2259	3195
Same parish	50 1	56 5	44 3	27 8	31 7	86 1	19 5	22 6	73 6
Same municipality differ parish	10 6	9 7	11 3	13 6	15 7	4 1	16 2	13 9	6 9
Same county differ municipality	16 1	14 2	17 8	22 0	23 2	4 9	24 3	24 0	9 7
Different counties	23 2	19 5	26 6	36 6	29 4	5 0	40 0	39 4	9 8
Degree of urbanization									
All	13663	6436	7227	1415	1962	3041	1721	2259	3195
Rural/Rural	3	5	2	2	2	7	2	1	3
Rural/Small city	1 0	9	1 1	1 8	1 3	2	1 7	1 7	4
Rural/City	3 9	3 7	4 2	6 6	5 7	9	6 1	6 1	1 8
Rural/Metropolis	1 9	1 7	2 0	2 7	2 9	4	2 4	3 1	1 0
Small city/ Small city	6 9	7 7	6 2	5 9	4 9	10 4	4 0	4 1	8 9
Small city/City	5 3	4 5	6 1	7 3	7 2	1 3	9 2	7 8	3 1
Small city/ Metropolis	1 1	8	1 4	1 0	1 5	2	2 4	1 7	7
City/City	43 6	45 3	42 0	39 6	41 7	50 3	37 6	38 2	47 2
City/Metropolis	5 5	4 6	6 3	7 8	6 9	1 5	9 3	8 8	2 8
Metropolis/Metro polis	30 5	30 5	30 5	27 1	17 6	34 0	27 0	28 2	33 9

Table 20 Concordance/discordance with regard to place of residence degree of urbanization All twin pairs

	Total			Men			Women		
	Tot	*Men*	*Women*	*26* 35	*36* 45	*46* 58	*26* 35	*36* 45	*46* 58
Place of residence									
All	18309	9064	9245	2063	2817	4105	2252	2896	3947
Same parish	47 8	53 9	41 8	27 0	31 7	83 3	18 9	21 7	70 8
Same municipality differ parish	11 2	10 5	11 8	14 9	16 0	4 5	16 5	14 3	7 5
Same county differ municipality	16 4	14 6	18 2	21 7	22 1	5 8	24 2	23 7	10 7
Different counties	24 6	21 0	28 1	36 4	30 2	6 3	40 5	40 3	11 0
Degree of urbanization									
All	18309	9064	9245	2063	2817	4105	2252	2896	3947
Rural/Rural	3	5	2	2	3	7	1	2	3
Rural/Small city	1 0	9	1 1	1 9	1 2	2	1 6	1 6	5
Rural/City	4 0	3 7	4 2	6 3	5 3	1 2	6 0	6 1	1 9
Rural/Metropolis	1 9	1 7	2 0	2 7	2 7	6	2 4	3 1	1 0
Small city/Small city	6 4	7 3	5 6	5 3	5 0	9 9	3 7	3 8	8 2
Small city/City	5 5	4 6	6 3	7 8	6 6	1 6	9 0	8 1	3 4
Small city/Metropolis	1 2	9	1 6	1 3	1 2	3	2 7	1 9	7
City/City	43 0	44 1	41 9	39 2	40 9	49 1	38 5	38 1	47 0
City/Metropolis	6 1	5 4	6 9	8 2	8 0	1 9	9 5	9 3	3 3
Metropolis/Metropolis	30 5	30 9	30 1	27 0	28 8	34 5	26 5	27 9	33 7

Table 21 (cont d) Concordance/discordance with regard to place of residence degree of urbanization Women

	Total			MZ			DZ		
	Tot	MZ	DZ	26-35	36-45	46-58	26-35	36-45	46-58
Place of residence									
All	6775	2694	4081	592	862	1217	1023	1267	1766
Same parish	44 4	49 6	40 9	21 5	28 5	78 8	18 5	19 2	70 0
Same municip differ parish	11 4	10 4	12 0	16 7	12 6	5 9	15 9	14 9	7 7
Same county differ municip	17 9	16 7	18 6	24 0	23 5	8 3	24 8	24 3	10 8
Different counties	26 4	23 2	28 4	37 8	35 4	7 0	40 8	41 6	11 5
Degree of urbanization									
All	6775	2694	4081	592	862	1217	1023	1267	1766
Rural/Rural	2	1	2		1	2	3	2	2
Rural/Small city	1 1	1 1	1 2	1 9	1 7	3	1 8	1 7	5
Rural/City	4 0	3 6	4 3	6 6	5 0	1 2	5 7	6 6	1 9
Rural/Metropolis	2 0	1 9	2 1	2 5	3 2	7	2 3	2 9	1 3
Small city/Small city	6 1	6 2	6 1	4 1	4 8	8 4	3 6	3 6	9 3
Small city/City	6 2	5 4	6 8	8 3	7 1	2 6	9 9	8 7	3 6
Small city/Metropolis	1 4	1 4	1 5	3 4	1 2	5	2 0	2 1	8
City/City	42 1	41 9	42 3	36 7	38 1	47 4	38 1	39 1	47 1
City/Metropolis	6 2	6 2	6 2	9 0	8 9	2 7	9 3	8 5	2 8
Metropolis/Metropolis	30 5	32 1	29 4	27 7	29 9	35 8	27 1	26 8	32 6

Table 21 Concordance/discordance with regard to place of residence degree of urbanization Men

	Total			MZ			DZ		
	Tot	MZ	DZ	26 35	36 45	46 58	26- 35	36- 45	46- 58
Place of residence									
All	5919	2273	3646	506	713	1048	807	1095	1735
Same parish	56 6	60 4	54 2	33 8	38 7	88 4	23 8	27 3	85 4
Same municip differ parish	9 7	9 5	9 8	13 8	14 9	3 8	13 7	16 3	4 2
Same county differ municip	14 3	13 2	15 0	20 6	22 2	3 6	23 7	24 2	5 2
Different counties	19 3	16 8	20 9	31 8	24 3	4 2	29 4	32 1	5 2
Degree of urbanization									
All	5919	2273	3646	506	713	1048	807	1095	1735
Rural/Rural	5	4	5	2	4	5	2	1	9
Rural/Small city	9	9	9	2 0	1 3	1	1 7	1 4	2
Rural/City	3 7	3 1	4 1	4 9	5 0	7	7 8	6 0	1 2
Rural/Metropolis	1 7	1 6	1 8	2 2	3 1	4	3 1	2 8	5
Small city/Small city	7 9	8 1	7 7	7 5	6 0	9 9	5 0	4 7	10 8
Small city/City	4 3	4 3	4 4	8 5	5 5	1 3	6 3	8 1	1 1
Small city/Metropolis	8	7	8	6	1 4	3	1 4	1 6	1
City/City	45 1	45 5	44 9	38 5	42 8	51 0	39 9	40 5	50 0
City/Metropolis	4 4	4 2	4 6	6 9	6 6	1 2	8 1	7 2	1 3
Metropolis/Metropolis	30 7	31 2	30 4	28 7	27 9	34 6	26 5	27 6	33 9

The tables appear in the following order in both the A-series and the B-series

Table 1: Personal data including specific twin questions

Table 2 Medical data

Table 3 Physical activity Height and weight

Table 4: Smoking habits

Table 5 Use of pharmaceuticals

Table 6 Drinking habits

Table 7 Personality variables and stress

Table 8 Food habits

Table 9 Annoyance reactions at place of work

Table 10 Annoyance reactions at place of residence

Table 11 Annoyance reactions other measurements

Table 12 Education occupation and work situation

Table 13 Residence situation sense of well-being etc

The tables are marked with the above number and header The tables belonging to the A-series bear the prefix A e g Table A1 Correspondingly tables of the B series are prefixed with a B The tables of the B-series are grouped into a men s and a women s section the men being presented first

Table 1 Personal data

The personal data are derived from questions 1 8 on the questionnaire Questions 1 and 5 being administrative control-questions are not tabulated Questions 2-4 are intimately bound to the pair-characteristics of the twins and are not included in the A-series

In the B-series married and not married (single divorced widow/widower) are regarded as mutually exclusive states Concordance married thus means the combination married-married Concordance not married means not married not married Discordance refers to not married married or married not married Number of children is also reduced to a nominal dimension have ch ldren do not have children

Table 2 Med cal data

Table 2 is a composite of the responses to questions 9 21 Chest pain occas onal in the A-series and chest pain: total in the B-series refer to responses to question 9 Repeated chest pain refers to the response to the first subquestion in question 10 From the construction of the questionnaire it follows that yes responses to question 10 comprise a subset of the yes response to question 9

Questionnaire data

The responses entered on the questionnaires are stored in the registry partly in coded form and partly as regular text Questions that are answered with a word or a sentence are stored as regular text while those answered by checking a box are assigned numerical codes Since a variable such as occupation contains many dimensions all of which cannot be expressed within a single code regular text is of more general use than a code Regular text however is more difficult to work with than a code as for example in tabulations The tables introduced below refer to all coded information questions stored as regular text are not presented

It must be stressed that the constructed tables are only examples classifications are made only arbitrarily The information stored in the registry is exactly as detailed as the questionnaire New tables with narrower or broader classifications can be produced Concepts may be given new definitions new variables may be generated

The explanatory text which follows is heavily condensed Since the respondents ways of answering a question are closely related to the phrasing of the questions it is essential to know exactly how the question is formulated and to some extent the order in which the questions are put The questionnaire in its entirety is presented in Appendix 1

The tables show the relative distribution of responses The base numbers have been inserted above the percentages as a rule on the uppermost part of the page The percentages can hence be re converted to absolute numbers by simple multiplication If the percentages are added the result will generally not be 100% due to the fact that certain respondents left the particular question blank For the sake of brevity the frequency of unanswered items is not taken up Unanswered items can be of two kinds Sometimes the respondent has forgotten or has been unable to answer the question i e internal non response In other cases the respondent has been instructed to leave certain questions unanswered since these are not relevant to him The difference between 100 % and the sum of the percentages is thus not always an expression of the internal non response The tables appear in two sequences the A series and the B series Notice also that since the A series contains all individuals that have answered the questionnaire while the B series contains all pairs where both twins have responded an individual distribution calculated from the B series will not be in agreement with the corresponding A series distribution

The A series has column headings for sex and age (expressed as year of birth) The B series is divided into sex zygosity and age (year of birth) Zygosity is broken down into MZ (monozygotic) and DZ (dizygotic) On the response to question 2 if both twins in the pair stated like two peas in a pod either alone or in combination with do not know" they were designated MZ If both responded no more alike than siblings in general either alone or in combination with do not know they were diagnosed as DZ All other combinations of answers were diagnosed as XZ which is not treated in this set of tables

do not exercise especially much and the other partner exercise rather much A group with a strong discordance as to leisure time exercise is then obtained by taking the difference between discordance and discordance 4/5

Table 4 Smoking habits

The answers to questions 26-34 in the questionnaire are tabulated in Table 4

Smoking status covers all types of tobacco taken up in the questionnaire hence also snuff/chewing tobacco

Smoking discordance in the B series table rests upon the same definitions as smoking status in the A-series table By concordant smokers is meant the logical sum of present smokers and former smokers i e the combinations present smoker-present smoker former smoker-former smoker present smoker former smoker The discordant group consists of the combinations present smoker-nonsmoker and former smoker-nonsmoker

Discordance with respect to quantity of cigarettes is expressed in terms of the absolute difference in the quantity of cigarettes smoked daily by the twins in a pair Observe that disc 10 cigarettes is a subset of disc 5 cigarettes

Abbreviations used in Table B4 are

S Smoker

NS Nonsmoker

CGT Cigarettes

Text of the type Disc pipe vrs not pipe means that one twin smokes a pipe wh le the other one does not The similar text "Disc pipe present vrs not pipe present should be interpreted in an analogous manner

Table 5 Use of pharmaceuticals

The answers to questions 35-40 are treated in Table 5 Question 39 is covered as part of the medical data (Table 1) in the A-series

Respondents who stated only almost never" on questions 35-38 are accounted for under the same heading in the A series table The same is true for those who stated only regularly for shorter or longer periods Under the heading now and then are presented those who filled in the "now and then alternative alone or in combination with regularly for shorter or longer periods The combination response regularly" plus almost never" and the combination of all 3 alternatives are not considered in the table

In the B-series regular usage of a given drug is set in contrast to the remaining responses Regular usage means that a twin filled in the answer regula ly for shorter or longer periods alone or in combination with now and then That the respondents filled in more than one answer however was unusual The first two responses were marked simultaneously in one hundred or so cases at the most Other combinations were not encountered in more than 20 cases in any of the questions

Angina pectoris (AP) is diagnosed from question 10 A positive AP-diagnosis calls for a yes response to the first subquestion in question 10 i e *experience of repeated pain or discomfort in the chest and traceability* of the pain to the middle or the left side of the chest (possibly combined with another of the alternative locations given in subquestion 10 d) Morevoer the pains must normally subside within 10 minutes (the other alternative in 10 c not acceptable) and the respondent *upon feeling the pains or* discomfort must stop or go more slowly and /or take medication but not continue at the same pace"

The diagnosis emotional angina pectoris is put forth if the described requirements are met and person responded that he feels the pain or discomfort when upset or overly stressed on question 10 a No other alternative on 10 a is acceptable for an emotional angina pectoris

The diagnosis effort angina pectoris is advanced if the requirements for angina pectoris is general are met and if on question 10 a the respondent says that he feels pain when he walks rapidly or walks upphill and/or walks at a normal pace on a flat terrain and not virtually at any time whatsoever or under other circumstances (If the respondent has checked upset or overly stressed in combination with "walks rapidly or walks uphill or walks at a normal pace on a flat terrain he is diagnosed as effort AP if the other requirement are fulfilled effort AP has precedence over emotional AP)

Table 3 Physcial activity and height and weight

Table 3 illustrates the replies to question 22 25 in the questionnaire Physical activity at work is rated numerically according to the following scale

Low 1

2

3

High 4

These ratings correspond exactly to the response alternatives so that low 1 is equivalent to mainly sedentary work and so forth The same applies to physical activity during leisure time

Relative body weight has been calculated according to the following algorithm

$$\text{Relative weight} \quad \frac{\textit{Weight in kg}}{(\text{Height in cm} \quad 100) \times 0\ 9}$$

Overweight is defined as relative weight exceeding 1 2

In the B series table the discordance index with regard to physical activity *is constructed by dichotomizing the scale near its midpoint Another index* for discordance has the notation 2/3 for activity at work and 4/5 for activity during leisure time These measurements of discordance mean that the respective twins responses fall on the response alternatives adjacent to the point at which the scale has been dichotomized *In the case of leisure time physical activity* the midpoint dichomotomization is between *those cases* in which one partner said

do not exercise especially much and the other partner exercise rather much A group with a strong discordance as to leisure time exercise is then obtained by taking the difference between discordance and discordance 4/5

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The answers to questions 26 34 in the questionnaire are tabulated in Table 4

Smoking status covers all types of tobacco taken up in the questionnaire hence also snuff/chewing tobacco

Smoking discordance in the B-series table rests upon the same definitions as smoking status in the A-series table By concordant smokers is meant the logical sum of present smokers and former smokers i e the combination present smoker-present smoker former smoker former smoker present smoker-former smoker The discordant group consists of the combinations present smoker-nonsmoker and former smoker-nonsmoker

Discordance with respect to quantity of cigarettes is expressed in terms of the absolute difference in the quantity of cigarettes smoked daily by the twins in a pair Observe that disc 10 cigarettes is a subset of disc 5 cigarettes

Abbreviations used in Table B4 are

S Smoker

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The answers to quest ons 35-40 are treated in Table 5 Question 39 is covered as part of the medical data (Table 1) in the A-series

Respondents who stated only almost never on questions 35-38 are accounted for under the same heading in the A-series table The same is true for those who stated only regularly for shorter or longer periods Under the heading now and then are presented those who filled in the now and then alternative alone or in combination with regularly for shorter or longer periods The combination response regularly" plus almost never" and the combination of all 3 alternatives are not considered in the table

In the B-series regular usage of a given drug is set in contrast to the remaining responses Regular usage means that a twin filled in the answer regularly for shorter or longer periods alone or in combination with now and then That the respondents filled in more than one answer however was unusual The first two responses were marked simultaneously in one hundred or so cases at the most Other combinations were not encountered in more than 20 cases in any of the questions

Table 6 Drinking habits

Table 6 displays the replies to questions 41-45 in the questionnaire The two first sub tables in the A series consist of a classification based on question 41 Total abstainers are those who answered no to all of the three subquestions or who stated 0 or when asked the number of bottles

Note that the alternatives are not mutually exclusive as regards distribution of consumption

The key to interpreting the first sub-table in the B series is the following Conc 000 means that both twins are total abstainers Conc 001 250 means that both have a monthly consumption within the range of 1 250 grams of absolute alcohol *and so forth Codes from 1 5 are assigned to the* concordance material and are utilized in the discordance material as well For example disc 1 vrs 3 means that the one consumes between 1 250 grams of absolute alcohol per month while the other between 501 750 grams

Table 7 Personality variables and stress

Table 7 on personality variables and stress covers questions 46 64 as well as 93 The emphasis is upon two variables the degree of introversion-extra version and the degree of instability These variables are expressed in scores obtained through a summation of responses to certain of the questions 46-64 In the summation every original question is considered to have the same weight The questions employed to determine the introversion extra version dimension are 46 50 51 54 55 57 61 63 The scores are obtained by counting the number of no responses for questions 50 57 and 61 and the number of yes responses for the remaining questions The lowest scores means introversion and the highest scores means extraversion Instability is based *upon the answers to questions 47 49 52 53 56 58 59 62 and 64* The scores are obtained by counting the number of yes responses A high figure corresponds to a high degree of instability Note that question 60 does not belong to either of these scales

As regards stress it should be observed that known cause of stress is a subordinate part of stressfilled daily existence

Table 8 Food habits

Table 8 shows the distribution of the answers to questions 65-69 Question 65 consists of a number of subquestions all of which have 5 identical response alternatives

1 Less than one time per month

2 One time or so per month

3 Several times per month or one time or so per week

4 Several times per week

5 Almost daily

With regard to the B series a conc *yes means* that both of the twins have

chosen response alternative 4 or 5   Conc -no means that any one of response alternatives 1 3 has been given by both twins   Discordance means that the one twin gave any one of the first three alternatives while the other gave any one of the last two alternatives   Pronounced discordance means that the one twin gave alternative 1 or 2 while the other twin gave alternative 4 or 5

Table 9 Annoyance reactions at place of work

Table 10 Annoyance reactions at place of residence

In Table 10 answers to questions 70 and 72 respectively are charted Questions 71 and 73 are not tabulated

Table 11 Annoyance reactions general measurements

Table 11 depicts the variables which have been constructed from questions 70 and 72 respectively   These variables are often used in studies of annoyance reactions

Annoyed at place of residence by any agent covers all persons who replied somewhat annoyed or very annoyed to at least one of the sub-questions in question 70   All persons who stated very annoyed" are presented under the next heading in the table   The distribution of the respondents who responded somewhat annoyed or very annoyed on 0 1 2 3-4 and so forth on the sub-questions is also tabulated

An analogous presentation has served for the annoyance reactions at the place of work

Table 12 Education occupation and work situation

This table displays questions 74 76 77 79 86

Table 13 Residence sit ation sense of well being etc

Table 13 refers to questions 88-92

The designation con house in the B-series refers to row-styled housing semi-detached housing or detached house

Under the head ng "number of rooms in the B-series Disc 2/5 gives the number of pairs in which the one twin s home has 0-2 rooms and the other twin s home has 6 or more rooms   Discordance alone refers to discordance with regard to the i tervals specified under concordance The number of persons residing together as shown in the B series should be interpreted in a like manner as the number of rooms

As for location of dwelling neighbours etc the term satisfied in the

Table 6 Drinking habits

Table 6 displays the replies to questions 41-45 in the questionnaire The two first sub-tables in the A series consist of a classification based on question 41 Total abstainers are those who answered no to all of the three subquestions or who stated 0 or when asked the number of bottles

Note that the alternatives are not mutually exclusive as regards distribution of consumption

The key to interpreting the first sub-table in the B series is the following Conc 000 means that both twins are total abstainers Conc 001 250 means that both have a monthly consumption within the range of 1 250 grams of absolute alcohol and so forth Codes from 1 5 are assigned to the concordance material and are utilized in the discordance material as well For example disc 1 vrs 3 means that the one consumes between 1 250 grams of absolute alcohol per month while the other between 501 750 grams

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As regards stress it should be observed that known cause of stress is a subordinate part of stressfilled daily existence

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Table 8 shows the distribution of the answers to questions 65 69 Question 65 consists of a number of subquestions all of which have 5 identical response alternatives

1 Less than one time per month

2 One time or so per month

3 Several times per month or one time or so per week

4 Several times per week

5 Almost daily

With regard to the B series a conc yes means that both of the twins have

TABLE A1 PERSONAL DATA

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	32374	15603	16771	3492	844	6922	4353	5330	7075
SIBLINGS APART FROM YOUR TWIN									
1-2	46 9	49 1	48.7	36.4	48.	55.3	39 8	7 3	51.7
3-4	20.0	28.	19.	23 1	28.6	19 3	28	19 5	18
5-	13 5	13.4	13.7	22 4	13.4	8.2	25.0	13 8	7.3
MARITAL STATUS									
SINGLE	38	44.0	33.	11 9	28.	77.8	9.3	12	64 3
MARRIED/CO-HABITATING	57 2	52 5	61	1	74.7	20.	82 0	8 5	34.1
DIVORCED	3 4	2.	3.9	5	4 8	3	6.	3 3	1 0
WIDOW/WIDOWER	4	2	6		2	1	2.8	3	1
CHILDREN, 0 CHILDREN	46.7	52.4	1.4	18 2	31.2	84.2	18.1	18 8	74.9
1-2	40 1	36.4	43.6	54.2	56.9	12.2	53	62.2	23 3
3-4	11 2	9.	12	23.6	11.0		26.	2	1.2
5-	1 2		1.5	3.2	3		4.5	1.0	
TWIN CHILDREN	1 0	8	1 2	1	1 0	1	2 5	1 5	3

TABLE A2 MEDICAL DATA

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-4	46-58	26-35	36-45	46-58
TOTAL	32	15603	1 771	3808	546	6921	435	5 30	707
CHEST PAIN									
OCCASIONAL	26 1	26.2	26.	29.	27 2	23.7	26.3	27 3	24.
REPEATED	12.6	12 5	12.4	15.	13 3	10.0	14.	1 8	10.4
ANGINA PECTORIS									
EMOTIONAL	2	2.8	2.	4.2	3 3	1 5	3.4	3.6	2.2
EFFORT		1		5	1	2	4	3	5
EMOTIONAL EFFORT	.2	8	3.4		3.	1.7	3.5	3.	2.6
SEVERE CHEST PAIN LASTING MORE 30 MIN	3.	8	3.3	5 4	4.0	3	9	3.	2 7
COUGH									
COUGH MORNING	6.	6.2	5	7 5	5.2	6.2	6.8	5 9	6.8
MORE THAN 3 MONTHS/YEAR	1	1.6	1.1	2.	1 3	1 8	1 5	9	9
PHLEGM MORE THAN 3 MONTHS/YEAR		1.0		1					6
BREATHLESSNESS	1 6	18.	2.7	14.2	10.5	4	23.	21 7	21.6
DISCOMFORT, PAINS, ACHES IN THE EXTREMITIES	2	15.8	22.	10.8	1	11.3	23	20.3	1 7
BACK DISORDERS									
PAIN IN THE BACK	1 8	16 8	13.	24.3	1 7	12.1	10 8	12	9.6
PAIN IN THE SHOULDERS	1	2.	3.	4.	3.2	1.6	1	3 6	1 7
PAIN IN THE BACK OF THE NECK	3	3.	4.8	4.	4.2	2.	6.5	3 1	2 3
MORE THAN ONE OF THE ABOVE	1 8	1 5	16.5	29.2	20	11.	2 9	1 7	11 8

B series covers all persons responding very satisfied or rather satisfied
'Not satisfied is composed of persons who were not especially satisfied or dissatisfied

TABLE I PERSONAL DATA

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-56	26-35	36-45	46-58
TOTAL	3237	15603	16771	3402	944	6121	35	5318	7075
SIBLINGS APART FROM YOUR TWIN									
1-2	48	49 1	48.7	38	48 4	53.5	39 8	47 3	39.7
3-4	20.0	20.6	19.	22 8	20.8	19 3	20	1 5	1
5-	13.5	13	13.7	22 8	13.	8.2	25.8	13 0	7.3
MARITAL STATUS									
SINGLE	39 6	44 8	31.	11	20.	77 8	3	12 6	64.3
MARRIED/CO-HABITATING	57 2	52	1	1	74.7	20 7	82.9	81 5	34.1
DIVORCED	3.	2.8	3.	5	4.8	5	6.	3 3	1 9
WIDOW/ER OTHER		2	6		2	1	2 8	3	1
CHILDREN, CHILDREN	44.7	52.	1.4	1 2	31.2	86.2	19 1	18 8	74.
1-2	40 1	36.4	3.	54.2	38.9	12 2	55.5	2 2	23 8
3-4	11.2	9.	12 9	23.8	11 8		26.5	1	1.3
5-	1 3	9	1 3	3 2	3		4.	1 8	
TWIN CHILDREN	1 8	8	1 2	1 8	1 0	1	2 5	1 8	3

TABLE II. MEDICAL DA

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-56	26-35	36-45	46-58
TOTAL	323	5603	77	3402	946	6921	353	333	7875
CHEST PAIN									
OCCASIONAL	2	26.	26.	29.4	27 2	23.7	26.5	27 3	4.
REPEATED	1	1	12.	15.9	13 3	18.0	1	11	10.
ANGINA PECTORIS									
EMOTIONAL	2	2.8	2.	4.2	3 5	1.5	3.4	3.4	2 2
EFFORT	3	3			1	2	4	3	5
EMOTIONAL EFFORT	3.2	3 8	3	4.	3.	1.7	3.8	3.	2
SEVERE CHEST PAIN LASTING MORE 30 MIN	7	4.8	3.3	5	4.	3 3	4.9	3.	2 7
COUGH									
COUGH REGULAR	6.5	6.2	5	7 5	5.2	2	6.8	9	6.8
SOME TIME MONTHS/YEAR	1 3	1	1 1	2.	1	1 0	1	9	
PHLEGM MORE THAN 3 MONTHS/YEAR	9	1 0	7	1					
BREATHLESSNESS	1	10.	23.7	14.2	8.5	.4	25.	21 7	21 6
DISCOMFORT PAINS ACHES IN THE STOMACH	1	15.	22.	18.	1	11.3	23.4	29 3	3 7
BACK DISORDERS									
PAIN IN THE BACK	1	16	13.	24.	1 7	12.1	18 9	12	9.6
PAIN IN THE SHOULDERS	3 1	2.	5.4	4.6	3.2	1	6.1		1 7
PAIN IN THE BACK OF THE NECK	4.	3.8	4.8	6.	4.2	1	8 6	5 1	2 3
MORE THAN ONE OF THE ABOVE	1	19.5	6.3	25.2	20.	11.7	2 9	14 7	11.8

[

B series covers all persons responding very satisfied or rather satisfied
"Not satisfied is composed of persons who were not especially satisfied or dissatisfied

TABLE A4 SMOKING HABITS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	32374	15603	16771	3402	446	6921	383	3320	7073
SMOKING STATUS									
NONSMOKER	4 1	34 8	49.1	33 5	32 8	1	57 8	45 1	44.
PRESENT SMOKER	43 6	3.6	37 7	48.3	53.	7.6	29 8	3 5	1 1
FORMER SMOKER	12	22 9	12 8	27	14.2	9 8	10 2	1	11 1
CIGARETTE STATUS									
PRESENT SMOKER	34 8	34 8	37 1	32 2	37 4	34.	29	39 0	40.4
FORMER SMOKER	13	19	12 8	23.9	22.9	14.	10.2	14 6	11 2
CIGARETTE QUANTITY									
PRESENTLY 1-5 PER DAY	6	5.9	6.6	5 7	6 2	8.8	3 3	4 2	8.4
6-10	23 6	22 3	25.8	8 3	18 8	13.8	11 5	19.6	18 7
11-15	2	7	8 7	6.	8.7	7	7 4	10	8 3
16-20	3	8.0	4.8	9	10.0	6.2	4.2	8	4.2
21-	1.8	1 5		2 1	2 2	8	4	8	8
AGE AT START OF CIGARETTE SMOKING									
PRESENT AND FORMER 5 YRS	1 1	17 3	11 2	7 5	16.1	23.	1 4	6 5	20 6
16-20	28.8	29 3	28.2	39.	33.3	22 1	33 3	33 9	36.7
21-25	1	3.5		1	6	8	8 3	5 9	1 3
26-30	1		1	1 3	8		3 2	1 6	1
31-	9	3		1 2	1		2.8		
AGE AT STOP OF CIGARETTE SMOKING YEARS			5		1	1 7		1	1 2
16-20	3	3	2	1.8	2 2	6.2		1 1	4.
21-25	4.2	5.	2.6	2	8 7	5.8	1 3	3 8	
26-30		4.	2.	4 5	9	5	2 2	3	3
31-35	1	2 5		4.6	4.5		2 2	2	
36-	1	2.	1.2	10.	3			3	
FILTER									
WITH FILTER	2.1	39.6	44.3	24 5		42.5	31	6.1	44 7
WITHOUT		11	3 8	22	14.3	4.3		7	9
WITH AND WITHOUT		5	1	3	2				1

TABLE A4 SMOKING HABITS CONT

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	321	15603	1 771	3402	4444	921	353	53 0	7073
CIGAR/PIPE/CIGARETTE STATUS									
PRESENT SMOKER	8 5	9 0	2 2	2		8.3	2 4	2.6	2.2
FORMER SMOKER	2		7	3	5.8	4.4	7	6	7
CIGAR/CIGARILLO/CHEROOT QUANTITY									
PRESENTLY 3 PER DAY	1.		8	2 1	2.8	2.3	2	3	3
4-6			3	1 2	2 2		2		3
7-9	1	2	1		1	3	1	2	
10-		1.8		1 1			3	3	3
3-	6	1.8	3		1	7	1	5	2
TYPE OF TIP									
PRESENT SMOKER		2 1	7	21 3	28.7	16 7	3	8	1 1
FORMER SMOKER	.6	13 2	9	3.6	13 8	22.		3	8
QUANTITY OF PIPE TOBACCO PRESENTLY PACKAGE/WEEK		13.1	3	23	18.0	16.7			
2-	1 8		1	2.7	2 1	1.3	2	2	
4-		3		1	1	2		1	1
6-				1	2	1			2
SNUFF/CHEWING TOBACCO STATUS									
PRESENT USER		13 8	2	3	1 1	13.9	1	1	6
FORMER USER	8	3.6	1	2 6	3.3	4.			2
SNUFF/CHEWING TOBACCO QUANTITY PRESENTLY BOXES/WEEK	3		1						
2-	2.8	4.		4.	7	8.3			1
4-5		5		2.	4.2	4.			1
6-		2		3	7	5			1
				1	1	2			
TYPE OF TOBACCO									
ONLY CIGARETTES	3-	16.8	2.8	18.5	18.0	15.8	33.2	44 8	3 9
ONLY CIGAR/CIGARILLO/CHEROOT				1	9	7	3	3	
ONLY PIPE		1		3.6	2 2	8			
ONLY SNUFF/CHEWING TOBACCO	5	3.2		4	3	4.8			1

TABLE A2 MEDICAL DATA CONT D.

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	32374	15603	16771	3502	4866	6921	353	5330	7075
HEADACHE									
SEVERE HEADACHE	10	7 3	14 2	9 5	8.8	5 8	16 6	15 4	11 9
HEADACHE VISUAL DISTURBANCES/ VOMITING	4 8	2 5	6 8	3 6	3 0	1 6	9	7 7	5
IMPAIRED HEARING	7 3	10 1	4 7	16 9	9 5	6 7	6	3 8	4 1
ALLERGIES	16 6	14 8	18 2	12 4	15 0	16 0	17	18 1	18 6
CAUSE KNOWN	7 7	6.5	8 9	5 1	6.9	7 0	9 3	8 9	8 7
COLDS									
0 TIMES/YEAR	10.5	12 1	8.9	18 3	12 4	8 5	15 7	8 2	5 3
1	43 1	44 6	41 7	51 3	48.9	37 9	51 0	4 2	14 0
	29 8	28.0	31 4	20 7	27 3	32 6	22 5	31 9	36
3	9 3	7 4	9 2	3 4	5 2	11 0	3	7 5	13 7
4	5 3	4 6	5 9	1 8	3 5	6 9	2 1	9 3	8.8
LONG-LASTING OR SERIOUS ILLNESS	15 2	15 6	14 7	22 0	16.3	11 7	21 4	16 2	10.9
SICK LEAVE FOR OVER 3 CONSECUTIVE MONTHS	10 3	11 1	9 5	18 5	14.1	5 0	17 2	10 7	4 0
REGULAR INTAKE OF SOME PHARMA-CEUTICAL	11 3	9 3	13 1	12 0	9 0	8 1	16	13 3	10 4

TABLE A3 PHYSICAL ACTIVITY AND HEIGHT AND WEIGHT

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	32374	15603	16771	3802	4866	6921	4353	5330	7075
PHYSICAL ACTIVITY AT WORK									
LOW 1	32 8	33 3	32 3	29 8	29 7	38 4	19 9	22 6	47 2
2	19 7	16.4	22 8	20.4	19 6	12 0	28 5	27 3	15.8
3	37 5	36 3	38.5	35 4	36.8	36 4	3 4	44 6	30 8
HIGH 4	6 6	11 3	2 2	13 0	12	9 8	3	2 1	1 4
PHYSICAL ACTIVITY DURING LEISURE TIME									
LOW 1	11 8	11 4	12 1	11 4	11 9	11 0	11 2	12 6	12
2	11 4	9 6	13.2	10 4	10 6	8 5	13.9	1 6	31
3	15 7	1 2	17 1	15 9	15 6	12 3	17 2	18 0	18 5
4	29 8	26.3	31 2	31 1	26.7	23 5	33 0	31 6	29 7
5	21	22 2	20 6	21 1	22 2	22 7	20 3	19 4	21 7
6	6.5	9 5	3.7	7 0	7 8	12 1	3 0	2 3	5 1
HIGH 7	3 6	6 1	1 3	2 3	7	9 3	6	8	2 0
HEIGHT									
150 CM	7	2	1 1	1		3	1.6	1 1	8
151 160	14 0	1 4	29 4	3 1	4	2 3	3 0	2 2	21
161 170	38 4	15.4	40.6	20 0	13 7	14 8	16.5	62 7	61 9
171-180	31 7	52 5	12 4	55 8	55 3	8 7	7	12 0	13
18	14 2	29 2	1	21 9	24	33			3
WEIGHT									
-40 KG	4	3	5			8	2	2	9
41-60	42 7	13 5	69 9	5 7	6 8	22	52	70 6	80 2
61-80	47 6	69 5	27 1	65 7	73 6	67 1	2 9	27 2	17
8 100	8 0	14.9	2 6	23 5	18 1	8 0	3 5	1 3	
101		7	1	1 2	9	3	2	3	
RELATIVE WEIGHT									
-0.80	3 6	3 0	4.3	4	8	6.8	9	3 1	7
0 81-1 00	50	43 8	54 9	21 7	33.	61 8	33 3	57 6	71 0
1 01 1.20	36 3	3	29.7	5 0	53 6	27.4	2	31 8	17
1 21 1 30		7 9	4.8	16	8 7	2 7	19	5 7	2 0

TABLE 44 SMOKING HABITS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	3237	15603	1 771	3602	866	6921	4393	5310	7075
SMOKING STATUS									
NEVER SMOKER	43 1	36.8	49 1	33 9	32 8	41.9	37 8	5.1	44.
PRESENT SMOKER	43 4	41.6	37	48 3	53.	47 6	29 8	39 5	1 1
FORMER SMOKER	12.4	12 9	12.4	17 4	14.2	9 6	10 2	1	11 1
CIGARETTE ST TUS									
PRESENT SMOKER	34 0	34.8	37 1	32.2	37	34	29	39 8	40.4
FORMER SMOKER	13.4	19	12 8	23 9	22 9	14.6	10.2	1 .6	11.2
CIGARETTE QUANTITY									
PRESENTL 1- PER DAY	6	5 9	6.8	5 7	4.2	5 8	5.3	6.1	4
6-10	13.6	11.3	15.8	3	10.0	13 8	11 5	15.4	16.7
11-15	2	7 6	8.7	.4	8.7	7	7 4	10 5	3
16-20	4.3	8.0	4.8	8 5	10 8	6.2	2	4 8	4.2
21-	1.0	1 5		2.1	2 1		6	3	5
AGE AT START OF CIGARETTE SMOKING									
PRESENT AND FORMER 5 YEARS	1 1	17 3	11.2	7 8	1 1	13	(	5	20
16-20	24.8	3	28.2	35.	35 3	22.1	21 2	35	26 7
-25	4.1	3.9	4.	1	4.	8	8 3	5 9	1 8
26-30	1 8		1	1 5	8		3.2	1 6	1
31-	5			1 2	1		2 0		
AGE AT STOP OF CIGARETTE SMOKING									
5 YEARS		8	5		1	1 7		1	1.2
16-20	3 0	3.4	2	1 0	2 2	1		1 1	4.
21-25	4.2	5.0	3.6	2	8.7	9.8	1 3	3	8
26-30	3	4.3	2.	5	9 3	5	2.3	4.	3
31-35	9	2 3	2	6	4.3		2	2 9	
36-	9	2 7	1 2	10	3			3	
LTER									
ITH TER	2 1	38.8	44.3	26 5	44	2 5	31 1	9.1	4 7
TTHOUT		11	3.8	22.	14.1	4.3	7	7	
TH AND WITHOUT	3	8	1	3	2				1

TABLE 44 SMOKING HABITS CONT'

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	3237	18403	1 771	3802	4866	6921	43 3	5 10	7075
CIGAR/CIGARILLO-ARETTE ST TU									
PRESENT SMOKER	5 5		2 2	2	9	8.3	2 9	2.4	2.2
FORMER SMOKER	2				5.8	4.4	7	6	7
CIGAR/CIGAR-CIGARETTE QUANTITY									
PRESENTL 1-3 PER DAY	1 3	2 1	3	1	2 5	2.3	2	3	3
4-6		9	3	1.1	1 2	7	2		3
7-9	1	2	1	2	1	3	1	1	
10-12		1.8		1	9		3	5	3
13-		1 8	3	6	1	7		5	2
PIPE STATUS									
PRESENT SMOKER	5	1 1	7	21 3	20.7	16 7	3	3	1 1
FORMER SMOKER	.6	13 2	5	1	15 9	11 5		3	
AMOUNT OF PIPE TOBACCO									
PRESENTL PACKAGE/WEEK		12 1	3	13	11.0	10 7	2	2	
2-3	1 8	9	1	2	2 1	1 3		1	1
4-5	1			1	1				1
6-	1			1	2	1			
SNUFF/CHEWING TOBACCO ST TUS									
PRESENT USAGE		13 5	2	3	14.1	13.	1	1	
FORMER USAGE	1	3.6	1	1	3 3	4.9			2
SNUFF/CHEWING TOBACCO QUANTITY									
PRESENT BOXES/WEEK	3	7	1	4.8	7 9	8 3			
2-	2	4.		2.4	2	4.9			1
4-5		5		3	7	5			1
6-	1	2		1	1	2			
TYPE OF TOBACCO									
ONL CIGARETTES	24	16.	2.8	18.5	18.8	15.8	33.1	44 8	8 9
ONL CIGAR/CIGAR-CIGARETTES				1	9	7	3	3	
ONLY PIPE		1		.6	2 1	8			
ONL SNUFF/CHEWING TOBACCO	5	3		5	3	4 8			1

TABLE A5 USE OF PHARMACEUTICAL

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26–35	36–45	46–58	26–35	36–45	46–58
TOTAL	32374	15603	16771	3802	4866	6921	4353	5330	7073
RESTORATIVES									
ALMOST NEVER	59 7	70 3	47 9	72 9	72 3	68.4	46 3	46 8	49 7
NOW AND THEN	26 3	18 5	33.5	14 7	17 6	20 1	34 5	34 1	32 4
REGULARLY DURING CERTAIN PERIODS	10.2	5.1	15 0	4 1	4 4	6.2	13 5	15 7	15.3
PRESCRIPTION-FREE ANALGETICS									
ALMOST NEVER	63 0	71 4	55 1	67 3	68 1	76 0	51 5	54 1	58 1
NOW AND THEN	28 7	20.4	36.5	22 7	24 3	16.4	38 3	37 5	34 6
REGULARLY DURING CERTAIN PERIODS	1 5	8	2 1	1 5	9	5	2 2	2 4	2.0
SLEEPING PILLS									
ALMOST NEVER	85 6	86 8	84 4	83 3	86 9	88 8	77 9	84 6	88 4
NOW AND THEN	3 2	2 1	4.1	4 0	2 1	1 1	7 0	4 3	2 3
REGULARLY DURING CERTAIN PERIODS	1 1	8	1 5	1 3	8	5	2 3	1 5	9
TRANQUILIZERS									
ALMOST NEVER	81 4	86 2	78 9	80 1	83 8	86 8	78 7	77 6	84 9
NOW AND THEN	6 7	4 3	9 0	7 0	4 6	2 6	14 2	10.4	4 7
REGULARLY DURING CERTAIN PERIODS	3 3	2 4	4.2	3 4	2 6	1 6	5 7	4 6	2 9
ORAL CONTRACEPTIVES PRESENT									
USE YES			17 6				9 2	20 1	20.9
NUMBER OF YEARS 3			9 8				3 4	8 7	14 6
4–6			5 4				3 1	7 2	3.3
7–			2 1				2 3	3 9	6
ORAL CONTRACEPTIVES FORMER									
USE YES			28 0				17 1	38.2	27 1
NUMBER OF YEARS 3			23 5				14 1	31 6	23 3
4–6			3.7				2 2	5 4	3.2
7–			6				6	9	3
NEVER TOOK ORAL CONTRACEPTIVES			44.1				56 4	36 0	47 2

TABLE A6 DRINKING HABITS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26–35	36–45	46–58	26–35	36–45	46–59
TOTAL	32374	15603	16771	3802	4866	6921	4353	5330	7079
TEETOTALLER	29 3	21 8	36 4	20 5	13.3	23.4	43	31 5	35.3
BEER CONSUMERS	55 5	67 3	44 5	65 1	75 2	63 1	36 4	49 3	47 3
WINE CONSUMERS	44 7	43 7	45.7	42 2	51 2	39 3	39 5	51	45 3
LIQUOR CONSUMERS	4 9	59 9	33 0	61 3	69 3	52 5	26.4	3 6	35 7
COMBINATION OF TYPES OF BEVERAGE									
ONLY BEER	5 6	5 8	5 4	5 7	5 4	6.2	4 0	5 6	6.0
ONLY WINE	3 4	1 2	5.5	1 3	1 2	1 2	6 3	6 8	4 7
ONLY LIQUOR	2 3	2 9	1 7	3 9	3	2 0	1 6	1 4	1 9
BEER WINE	6.3	3 9	8.5	3 2	3.9	4 4	7 0	10 4	8 8
BEER LIQUOR	6 4	10.8	2.4	10 8	11 5	10.2	1 6	2 2	3 8
WINE LIQUOR	3 3	2 1	4 4	2 9	2 7	1 1	4 3	4 6	4.3
BEER WINE LIQUOR	27 6	34 1	21 5	32 3	40 8	30 5	16 5	24 8	22.8
BEER, QUANTITY									
1 0 BOTTLES/MONTH	42 7	49 3	36.5	45 2	56 5	46 6	26 8	41 4	38 7
11 20	5 1	8 7	1 7	3	9 0	8 1	2 8	1 9	1 4
21 30	2 0	3 8		2	7	3 0	5		3
31–	1 0	2 0	1	2 4	2 0	1 7	1	1	2
WINE QUANTITY									
1 0 BOTTLES/MONTH	23 8	24 6	22 9	21 9	29 9	22 9	18 3	25 3	23 9
1 2 0	6 8	7 2	6 4	7 8	8 9	6 1	5 2	7 7	0
2 1–3.0	1 7	1 8	1 6	1 5	2 1	1 7	1 2	1 9	1 6
3	3 3	3 8	2 7	4.8	4.1	3 1	2 9	3.4	2.2
LIQUOR QUANTITY									
0 BOTTLES/MONTH	27 9	38 9	17 7	37 5	46 5	3	13 2	18 6	19 8
1 0	5	8 6	8	3	1	7 0	6	1 0	8
2 1 3.0	9	1 7	1	1 8	1 8	1	2	1	2
3 1	2 3	2 5	2	3 1	2 7	2 0	2	2	3
DISTRIBUTION OF CONSUMPTION									
HOLIDAYS AND WEEKENDS	25 5	33 0	18 5	29 4	33 8	3 2	16 1	19 8	19 1
THROUGHOUT THE WEEK	3.1	4 1	2 2	5 5	7	2	2 2	3 0	1 7
ONLY ON INDIVIDUAL OCCASIONS	4 3	1 5	50.7	5 2	7 2	35 4	2	52	82 3

TABLE A1. [illegible] (CONT'D)

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26–35	36–45	46–55	26–35	36–45	46–55
TOTAL	32174 15403	14771		3802	464	4921	4393	5310	7073
GRAMS OF 100% ALCOHOL/MONTH 1–250	25.1	22.7	27	22 3	2 2	20 6	21 1	30 4	28.6
251–500	12 3	17 6	7 8	15	19	1 .2	4.2	3	7
501–750	4.3	7	1.5	7 1	6.3	6.8	1 4	1 7	1 9
751–1000	1 7	3.		3 2	3 3	2 8	4	5	
1001–	1 6	3.1	3	3	3 3	2 3	3	2	3
MORE THAN HALF BOTTLE OF THE SAME OCCASION	11 5	19 5	4.0	12 1	16.8	28.6	2 9	2.7	2
EARLIER HIGHER CONSUMPTION	11 6	18 1	5.9	15 9	19 9	18.3	1 7	3	9.3
IF HIGHER CONSUMPTION EARLIER, HIGHEST QUANTITY									
BEER QUANTITY 1–10 BOTTLES/MONTH	3 7	9 1	2.	3.	3.	3.6		1 5	4.2
11–20	1	3.1	7	1	2	.3	1	3	1 1
21–30	1 1	1 5	3	1 0	2 0	2.	1	1	3
31–	1 1	2	2	1	2 6	2.8		1	3
WINE QUANTITY .8 BOTTLES/MONTH	1 8	2 3	1 2	1	2 3	2	5	9	1
1–2.0	1.2	1.6	7	1 8	1	1 8	3	4	1.2
3.0		8		3	7	1 1	1	2	
3.1–	1	2	1 0	1.1	2.	2			1
[illegible] TY 1.0 BOTTLES/MONTH	3.4		2 1	7	8.1	.4	7	1.4	3.5
1–2.0	2.	4.2	7	3 5	4.5	8	2	2	1.4
2.1–3.0	1 1	2	2	1 5	2 3	1		1	
3.1–	2 5	5.8	2	.2	6.4	4.	1	2	

TABLE [illegible] PERSONALITY [illegible] AND [illegible]

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26–	6–45	46–	26–5	36–5	46–5
TOTAL	323	1548	1 771	3802	4464	4921	393	5310	7073
[illegible] TY SCORE 0–4	75 9	82.2	78.1	82 0	83 1	81.6	72 7	71 8	7 3
5–9	3	3	25	1 1	1	18.	26.7	27 7	32.4
SCORE	1 7	22.	11.	25 2	23.5	21.8	3	1 .4	9 8
1–2	34	37.6	1 8	36 8	35.8	7	1	32 2	29
3–4	24.	2	24.1	20	20.8	23	24.4	23 3	27 9
5–6	1	1	16.8		1 1	1 9	15 3	15.4	1 9
7–8	7	8.	16.3		5 2	3.	6.9	10 1	11 2
	1	1	2.3	1 5	1	9	2.5	2 2	2.3
[illegible] SCORE 0–4	3.	37 1	4.8	1	39 2	33 0	51	52 5	44
5–9	8 7	62.3	49 6	57	60.2	66	7 3	46 9	52
SCORE	3.	1.	3.3	2	1 7	1.2	3	3	2 5
1–2	1 3	18.8	17	12	12 4	8.8	18.7	1	13.4
3–4	2	2	29 0	27.8	28 1	23.0	29 3	29 2	28.7
5–6		3	29.1	20	31 5	31.3	28 9	7	30 3
7–8	2	26.	1 5	24	24 9	2 .4	1	17	28.
		4.	2.	3.	3		2.0	1.8	2 5
COMB. OF [illegible] AND [illegible] 5–9,0–4	3	5	18.7	18.8	9.1	9	19.1	18.4	19.2
5–9 5–9	2	7	18.	6.	7	8.5	8	9 1	13.1
0–4 5–9	29	27	1 8	31	30.8	21.	33 7	33 8	27 3
0–4,0–4	46	56	38 9	50 3	2.8	57 8	38 7	37 8	39
DIFFICULTY IN FALLING ASLEEP	1	1 4	21 5	14.3	16.	18.6	20 1	1 7	4.4
STRESS-FILLED DAILY EXISTENCE	15 7	18 2	13.	22 1	20.	15	14.1	12 8	13 9
CAUSE OF STRESS WORK	11.1	13.	9.1	16.	1 3	19.2	10 5	8	.2

TABLE A7 PERSONALITY VA IABLES AND STRESS CONT D 1

	TOTAL	TOTAL MEN	TOTAL WOMEN	MEN BORN 26 35	MEN BORN 36-45	MEN BORN 46 58	WOMEN BORN 26 35	WOMEN BORN 36-45	WOMEN BORN 46-58
TOTAL	32374	15603	16771	3802	4866	6921	4353	5330	7073
EPI-Q ITE 1 9									
1 DO YOU LIKE HAVING LOT OF THINGS GOING ON AROUND YOU?	48 9	45 8	51 8	36.8	43 1	52 7	40.1	48 7	61 3
2 ARE YOU OFTEN UNEASY AND FEELING THAT THERE IS SOMETHING THAT YOU WANT WITHOUT KNOWING IT?	24.4	20 0	28 4	15 7	18 9	23.1	22 3	26 2	33 9
3 DO YOU ALMOST ALWAYS H VE AN ANSWER READY WHEN SPOKEN TO?	64 4	70 6	58 7	74 9	71 8	67 4	63 6	59 8	53.5
4 ARE YOU SOMETIMES HAPPY OR SOMETIMES SAD WITHOUT ANY SPECIAL REASON?	50 0	36.7	62.4	35 5	36.0	38 6	52.3	61 3	69 9
5 DO YOU PREFER TO KEEP TO THE BACK GROUND IN THE COMPANY OF OTHER PEOPLE?	32 2	28 0	36.1	33 2	30 1	23.7	42 9	38.4	30.1
6 DO YOU REGARD YOURSELF AS HAPPY AND CAREFREE?	58 7	62 8	54.9	62 0	63 3	63.0	55 9	55 1	54.2
7 DO YOU OFTEN REACH DECISIONS TOO LATE?	19 9	18 7	20.9	19 9	18.4	18.3	22.3	19 2	21 4
8 DO YOU OFTEN FEEL TIRED AND LISTLESS WITHOUT ANY SPECIAL REASON?	24 2	18 3	29.6	17 9	18 0	18.8	27 3	30.2	30.4
9 DO YOU HAVE A LIVELY MANNER?	47 1	45 9	48 3	47 0	43 6	46.9	48 6	45 5	49 6

TABLE 7 PERSONAL TY VA IABLES AND STRESS CONT D 2

	TOTAL	TOTAL MEN	TOTAL WOMEN	MEN BORN 26 35	MEN BORN 36-45	MEN BORN -58	WOMEN BORN 26-35	WOMEN BORN 36-45	WOMEN BORN 6 58
TOT L	32374	15603	16771	3802	4866	6921	4353	5330	7073
EP TEM 10-18									
10 CAN YOU QUICKL DESCRIBE YOUR THOUGHTS WORDS?	59 1	65 9	52 8	68 3	68.0	63.2	58 9	55 4	47 1
ARE YOU OFTEN LOST YOUR OWN THOUGHTS?	37 4	37 0	37 8	34 4	35 8	39.3	32 3	32 6	45 1
12 DO YOU HAVE ANYTH NG A' INST SELL NG TH GS OR AS I G PEOPLE FOR MONEY FOR SOME CHAR TABLE PURPOSE?	50 4	47 5	53 1	50 9	50 4	3 7	54 5	57 8	9 3
13 ARE YOU EXTREMEL SENS T VE ANY RESPECTS?	1 6	31 9	30 6	37 8	31 8	20 8	52 1	50 9	9 7
1 ARE YOU EVER TOO RESTLESS TO S T ST L ?	31 3	32 9	29 8	25 2	31 0	30 5	20.7	24 5	39 6
5 DO YOU KEEP THINGS TO YOURSEL EXCEPT TH GOOD FR ENDS?	64 8	60 8	68 6	62 3	62 5	58 7	69 7	68 3	68 3
6 DO YOU HAVE NY ENEMIES ARGUMENT?	15 1	12 1	17 8	19 0	11 9	10 7	21 6	19 2	14.5
17 DO YOU L KE TO CRACK JOKES AND ELL FUNNY STOR ES TO YOUR FR ENDS?	44	60.7	3 9	53	56 1	67 8	28.4	29 5	7 7
8 DO YOU CALL ... STEP ... THE S ... ?	37 6	27 8	6.7	2 7	27 2	27 1	9 5	8 1	3 8

TABLE AL. FOOD HABITS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	25-35	36-45	46-55	25-35	36-	46-55
TOTAL	3237	18603	16771	3602	866	4921	853	5310	7075
BROILED OR DEEP-FRIED FOOD									
TIME/MONTH	36.9	26.9	40.4	37.4	30.4	33.2	45.6	41.4	36.3
ONE TIME OR SO/MONTH	32.7	31.8	32.6	29.4	33.1	34.3	27.3	32.2	34.1
1 SEV TIMES/MON OR ONE TIME OR SO/WK	19.0	21.	14.9	14.0	21.2	29	10.1	1.7	18.1
SEVERAL TIMES/WEEK	5.2	7.2	3.3	3.	4.	9.3	2	3.3	3.7
ALMOST DAILY		1.2	.9	.9	1.1	1.3		7	
PAN-FRIED OR ROASTED FOOD									
1 TIME/MONTH				.6	.9		1.1	.7	.9
ONE TIME OR SO/MONTH	3	3.9	3.9	3.	.9	1	2	2.8	
SEV TIMES/MO OR ONE TIME OR SO/WK	23.1	23.1	21.2	22.5	21	2	16.	2	27
SEVERAL TIMES/WEEK	53.8	52	51.	50	55.5	51.7	54.5	54.1	51
ALMOST DAILY	16.3	16.8	16.2	19.7	16.4	16.1	1.0	18.4	12.7
PORK MEAT									
1 TIME/MONTH	3.2	3.2	3.2	2	2	3.8	2.3	2.7	1
ONE TIME OR SO/MONTH	.5	7.2	.7	4.1	.9			.5	11.2
SEV TIMES/MO OR ONE TIME OR SO/WK	5	33.	34.2	31.8	3.1	33	29.2	31.9	39.2
SEVERAL TIMES/WEEK	44.1	45	44.3	4	41.3	3.2	33	50.5	38
ALMOST DAILY	.6	.2	6.1	.8			6.		.9
SAUSAGE OR HOTDOGS									
<1 TIME/MONTH	.8	3.3	6.1	.3	5.	2	4.	9.6	6.2
ONE TIME OR SO/MONTH	13.4	13.8	14.	15.	14.	11.	17	17.1	3.3
SEV TIMES/MO OR ONE TIME OR SO/WK	3.7	4.6	4.8	3.8	.7	2.3	3.		44
SEVERAL TIMES/WEEK	27	30.3	24.	26	29.8	34.3	24.	23.3	23.3
ALMOST DAILY	.3	.5	3.2	5.7	3.9	4.2	3.4	2	3.7

THIS ALTERNATIVE INCLUDES SEVERAL TIMES/MONTH

TABLE AL. FOOD HABITS CONT'D

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-56	25-	36-45	46-55
TOTAL	3237	5603	1677	3602	4046	21	351	330	7075
LIVER KIDNEY,BLOOD OR OTHER ORGAN FOOD									
TIME/MONTH	37	26	37	32	36	39.2	19	25.0	35.1
ONE TIME OR SO/MONTH	36.	37	34	38.2	38	36.	3	33.4	33.5
1 SEV TIMES/MO OR ONE TIME OR SO/WK	28.3	27	29.1	29	28.	26.	1.	32.5	2
SEVERAL TIMES/WEEK	4.8	4.2	1.3		.8	2	6.		3.8
ALMOST DAILY	.3	.3	.4	.3	.2	.3	.5	.3	.3
FISH									
TIME/MONTH		5.3	4.	1.7	.6	6.		2.8	5.6
ONE TIME OR SO/MONTH	1	19	11.	11.	13.9	14.3	.7	1.6	1.3
SEV TIMES/MO OR ONE TIME OR SO/WK	3.2	53.	3.	51	56.8	58.1	32.5	55.3	52.4
SEVERAL TIMES/WEEK	6.1	23.	28.	29	23.0	23.5	31.5	38	3.
ALMOST DAILY		.6	.8	1.0			.9	.5	.7
SHELLED SEAFOOD									
TIME/MONTH	53.7	8	51	55	51	59.3	51.	8.1	53.6
ONE TIME OR SO/MONTH	29.	27.1	29.7	29.1	30.	23.	27	31.	29
SEV TIMES/MO OR ONE TIME OR SO/WK	10	9.7	11.8		11.8	6.	11.4	3.2	3
SEVERAL TIMES/WEEK	.5	1	1.6		1	1	1	2	1.4
ALMOST DAILY			2			.3	.2	.2	.2
RICE AND RICE DISHES									
TIME/MONTH	50.8	33	28.1	31.3	33.	32.	24.	28.9	30.
ONE TIME OR SO/MONTH		30.6	32.1	30.5	29.1	1	3	30	32.5
SEV TIMES/MO OR ONE TIME OR SO/WK	6.	24.7	28.1	25.1	4.1	24	29.1	28.6	27.2
SEVERAL TIMES/WEEK	6.	6.	7	9.	4.3	7.8			
ALMOST DAILY						2.8	.7	0	7.8 1
FLOUR-BASED FOODS (PORRIDGE, DRY CEREALS, PANCAKES ETC)									
TIME/MONTH	11	18.	12.8	11.8	11	9.3	11.6	11.8	14.9
ONE TIME OR SO/MONTH	1	21.3	22.	30.	32	32.7	30.1	31.6	34
SEV TIMES/MO OR ONE TIME OR SO/WK	3.	3.	33.5	32	35.2	2.5	3.3	34.7	32.8
SEVERAL TIMES/WEEK		1.6	17.9	1.3	13.7	1.2	28.0	18.5	16.3
ALMOST DAILY	13.1	5.1	11.3	1.7	13.3	1.5	12.0	11.9	10.5

THIS ALTERNATIVE INCLUDES SEVERAL TIMES/MONTH

TABLE A7 PERSONALITY VARIABLES AND STRESS CONT D 1

	TOTAL	TOTAL MEN	TOTAL WOMEN	MEN BORN 26 35	MEN BORN 36-45	MEN BORN 46 58	WOMEN BORN 26-35	WOMEN BORN 36-45	WOMEN BORN 46-58
TOT L	32374	15603	16771	3802	4866	6921	4351	5330	7075
EPI-Q ITEM 1 9									
1 DO YOU LIKE HAVING A LOT OF THINGS GOING ON AROUND YOU?	48 9	45 8	51 8	36.8	43 1	52 7	40.1	48 7	61 3
2 ARE YOU OFTEN UNEASY AND FEELING THAT THERE IS SOMETHING THAT YOU WANT WITHOUT KNOWING IT?	24 4	20.0	28 4	15 7	18 9	23.1	22 3	26 2	33 9
3 DO YOU ALMOST ALWAYS H VE AN ANSWER READY WHEN SPOKEN TO?	64 4	70 6	58.7	74 9	71 8	67 4	63 6	59 8	53.5
4 ARE YOU SOMETIMES HAPPY OR SOMETIMES SAD WITHOUT ANY SPECIAL REASON?	50 0	36.7	62.4	34 5	36.0	38.4	52 3	61 3	69.5
5 DO YOU PREFER TO KEEP TO THE BACK GROUND IN THE COMPANY OF OTHER PEOPLE?	32 2	28 0	36 1	33 2	30 1	23.7	42 9	38.4	30.1
6 DO YOU REGARD YOURSELF AS HAPPY AND CAREFREE?	58 7	62 8	54 9	62 0	63 3	63 0	55 9	55 1	54.2
7 DO YOU OFTEN REACH DECISIONS TOO LATE?	19 9	18 7	20.9	19 9	18 4	18.3	22 3	19 2	21 4
8 DO YOU OFTEN FEEL TIRED AND LISTLES ITHOUT ANY SPECIAL REASON?	24 2	18 3	29 6	17 9	18.0	18 8	27 3	30 2	30.6
9 DO YOU HAVE A LIVELY MANNER?	47 1	45 9	48.3	47 0	43 6	46.9	49 6	45 5	49 6

TABLE A7 PERSONAL TY VA ABLES AND STRESS CONT D 2

	TOTAL	TOTAL MEN	TOTAL WOMEN	MEN BORN 26 35	MEN BORN 36-45	MEN BORN 6-58	WOMEN BORN 2 35	WOMEN BORN 3 -45	WOMEN BORN 6-59
	32374	15603	16771	3802	4866	6921	4353	5330	7075
TOTAL EP -Q TEM 10-18									
10 CAN YOU QUICKL DESCRIBE YOUR THOUGHTS WORDS?	59 1	65 9	52 8	66 3	68 0	63 2	58 9	55 4	47 1
ARE YOU OFTEN LOST IN YOUR OWN THOUGHTS?	37 4	37 0	37 8	34 4	35 8	39 3	32 3	32 6	45 1
12 DO YOU HAVE ANYTH NG AGA NST SELL NG TH NGS OR ASKING PEOPLE FOR MONE FOR SOME CHAR T BLE PURPOSE?	50 4	47 5	53 1	50 9	50 4	43 7	54 5	57 8	9 3
13 ARE YOU EXTREMEL SENS TIVE ANY RESPECT??	1 6	31 9	50.6	37 8	31 8	28 8	52.1	50 5	4 7
1 ARE YOU EVER TOO RESTLESS TO ST LL?	32 3	32 9	29 8	25.2	31 0	38.5	20.7	24 5	39 4
5 DO YOU KEEP TH NGS TO YOURSELF EXCEPT TH GOOD FR ENDS?	64 8	60 8	68.6	62 3	62 5	58 7	69 7	68 3	68 3
6 DO YOU VE ANY NERVOUS PROBLEMS?	15 1	12 1	17 8	15 0	11 9	10.7	21 6	19 2	14 5
17 DO YOU L KE TO CRACK JOKES O TE L FUNNY STOR ES TO YOUR FR ENDS?	44	0.7	36 9	53 6	56.1	67 8	28	29 5	7 7
18 DO YOU TOTALLY HAPPY AFTER D THE NG NT	5	27 8	44 7	29	27 2	27 1	9 9	8 1	3 8

TABLE 34. ANNOYANCE REACTIONS AT PLACE OF WORK

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-55	26-35	36-45	46-55
TOTAL	32374	13603	14771	3802	4864	921	4353	5338	7079
NOISE									
ANNOYED	23 7	34.1	12.1	31.5	37 5	32.0	3	10 6	15.1
VERY ANNOYED	1	1 5	3.1	10 9	13.3	14.5	2.5	2 3	3 7
VIBRATIONS									
ANNOYED	6 8	13 3	3.2	11.7	14.1	12.5	2 8	2.5	3 9
VERY ANNOYED	2 0	3.	4	3 5	3.	3.1	7		8
DIRT, SOOT, SMOKE OR GASES									
ANNOYED	1	27 1	9	26.2	31 2	2 8	8 3	7 9	11 2
VERY ANNOYED	5 7	8	2.6	8.2	11 1	8.0	2.3	2 1	3.0
ODOR ANNOYED	11 2	14.8	7 7	14.2	14.2	14.2	6.5	4 2	9 6
VERY ANNOYED	2.7	3.	1.7	3.		3.3	1.4	1	2.0
POOR/GLARING LIGHTING									
ANNOYED	10 8	12.5	7 7	12.2	13 9	11.8	6.3	6 8	3
VERY ANNOYED	2 8	2	1 4	2 3	3.7	2.2	1 1	1.4	1 7
HUMIDITY									
ANNOYED		7 8	5.	7 7	9.8	6.6	9.2	5	6 1
VERY ANNOYED	1.6	1 8	1 3	1.8	2 2	1.	1 1	1 9	1 3
INTENSE TEMPERATURE CHANGES									
ANNOYED	1	17 0	9 2	1 5	1 1	5.8	7 3	7.4	11
VERY ANNOYED	3 7	8 1	2 3	4.	6.2		2 1	1	2 8
TOO HOT ANNOYED	11	1	1	14.3	14.3	13 9	8 2	8 1	10.
VERY ANNOYED	3.	4.	2.3	0		4.5	2 4	2 3	1 3
TOO COLD, ANNOYED	10 1	13.2	2	1 7	14.4	12 0	5	5	6
VERY ANNOYED	6	8	2	4.8	4.0	3.5	1.5	1.	2 7
UNSUITABLE WORK POSTURES									
ANNOYED	14.	19.3	10.7	1 .3	1	10.3	.6	8 9	13.3
VERY ANNOYED	3	5.2	2 8	5	5.	5.0	2.2	2.2	2.9

INCLUDES VERY ANNOYED

TABLE 35 ANNOYANCE REACTIONS PLACE OF RESIDENCE

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-46	46-55	26 35	36-45	46-5
TOTAL	32374	13603	1 77	3802	4864	4921	353	5330	7079
TRAFFIC NOISE									
ANNOYED	1 2	0.8		1 1	10.6	10.2	10 7		9.8
VERY ANNOYED	2 8	2.0	1		2 1	2.	2 1	1	2.0
AIRCRAFT NOISE									
ANNOYED	5.1	5	4.7	4.5	3.8	8.0		9	4.9
VERY ANNOYED	8	9	7	1.4	3	9	9		7
INDUSTRIAL NOISE									
ANNOYED	1.8	2	1	3.4	2 1	2.3	1	1 3	1 4
VERY ANNOYED			3			6	3	3	3
NOISE FROM NEIGHBORS									
ANNOYED	2	8.3	8	6.		4.8	7 4	9	12.0
VERY ANNOYED	1	1.	1.	1 2	1.	1.	1.	1 3	2.2
ODOR									
ANNOYED			3	7 9	8 2	8.0	9.	8.7	9.
VERY ANNOYED	2		8	1.6	1 9	1.6	2.0	1 7	1 7
AUTOMOBILE EXHAUST/GASES									
ANNOYED		8.	8.	8.3	6.0	7 3	9 3	6.8	10 2
VERY ANNOYED	2.8		2.	1 7	1	1.	2.	1 5	2 7
AIR POLLUTION									
ANNOYED	8	5		7 9	8.	8.7	9 9	8 5	9
VERY ANNOYED	2 2	2.	2.4	1	2 8	2.1	2 3	1	2.
DUST, SOOT									
ANNOYED	8	8 7	12.8		9.7	7	11 9	12 2	11
VERY ANNOYED	2.6	2.1	3.8	2.8	2.3	2.	3 2	2.	2.3
WATER POLLUTION									
ANNOYED	.3	1	4.3	3	4.1	4.1	1	3.	3.8
VERY ANNOYED	1 2	1.2	1.2	1	1 3	1.8	1 1	9	1.6

INCLUDES VERY ANNOYED

TABLE A8. FOOD HABITS CONT'D, 2

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26 35	36–45	46–58	26 35	36–45	46 55
TOTAL	32374	15603	16772	3802	4866	6921	4353	5330	7075
EGGS AND EGG DISHES									
1 TIME/MONTH	3 6	3 2	3.9	2 7	3.1	3 6	3 4	3 0	8
ONE TIME OR SO/MONTH	11 6	12 3	11 0	11 9	11 0	13 4	9 3	9 6	13 1
1) SEV TIMES/MO OR ONE TIME OR SO/WK	32.7	34.0	31.5	33 2	35 2	33 6	30 4	29 9	33
SEVERAL TIMES/WEEK	35 2	34 4	36.0	34 1	35 2	34	37 4	38 9	32 9
ALMOST DAILY	14 9	14 1	15 7	15 3	14.1	13.5	16.3	16 7	14 6
VEGETABLES AND ROOT FOODS									
1 TIME/MONTH	1 8	2 7	9	2 3	2 2	3 2	6	6	1 3
ONE TIME OR SO/MONTH	4 3	5 9	2 8	4 0	5.4	6.2	2 9	2 3	3 1
SEV TIMES/MO OR ONE TIME OR SO/WK	14 1	17 2	11 2	18 6	16 8	16 7	12 9	10 7	10.6
SEVERAL TIMES/WEEK	30 4	33.0	28 4	32 8	32 7	33.3	31 4	27 9	27 0
ALMOST DAILY	47 7	39 4	55.5	37 3	41 6	38.9	50 4	57 5	57 1
FRUIT									
1 TIME/MONTH	1 4	2 0	9	3 3	2 1	1 1	1 1	8	7
ONE TIME OR SO/MONTH	3 2	4 8	1 6	6 1	5 5	3 3	2 4	1 5	1 3
SEV TIMES/MO OR ONE TIME OR SO/WK	7 8	11 2	4.6	12 4	10.9	10 7	5 0	4 3	7
SEVERAL TIMES/WEEK	22 2	28.6	16.2	28 3	28 7	28 7	16.7	15 8	16 2
ALMOST DAILY	63 9	51 6	75.4	46 8	51 4	54 4	72 5	76 6	76 2
MILK, SOUR MILK, YOGURT OR CHEESE									
1 TIME/MONTH	8	9	6	1 2	9	7	9	4	7
ONE TIME OR SO/MONTH	7	9	5	1 1	1 0	8	5	4	6
SEV TIMES/MO OR ONE TIME OR SO/WK	2 3	3 1	1 4	4 0	3 1	2 6	1 9	1 5	1 6
SEVERAL TIMES/WEEK	5.2	6 1	4.4	7 2	6 5	5 1	4 8	4 1	4 4
ALMOST DAILY	90 0	87 8	92 0	84 6	87 7	89 8	90 7	92 9	92 1

1) THIS ALTERNATIVE INCLUDES SEVERAL TIMES/MONTH

TABLE A8 FOOD HABITS CONT D 3

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26 35	36–45	46–58	2 35	36 5	-5
TOTAL	32274	15603	1 771	3802	4866	6921	4353	5330	7075
WARM MEALS PER DAY									
0 MEAL(S)	1	1	2	1	1	1	1	1	3
1	39 2	33 0	44.9	34 2	41 4	25 4	41 8	47 4	9
2	51 2	55 0	47 7	54 0	52 4	57 3	50 5	46 2	47 1
3	7 8	9 7	6.0	7	4 7	1 3	6	3 4	2
4–	4	4	3	2	1	1 1		1	5
SANDWICHES PER DAY									
0 SANDWICH(ES)	1 1	7	1 4	1 0	8	5	2 1	1 5	1 5
1 2	22 4	11 0	13.0	13 1	12 3	8 9	34	34	30 7
3–4	39 8	31 7	47 3	34 6	34 8	27 9	7 9	8 0	46 5
5–6	23 0	32 2	1 4	31 8	33 7	33 5	13 0	13 1	16.2
7	12.3	22 5	2 7	17 1	20.0	27 2	1 8	1 7	0
COFFEE PER DAY									
0 CUP(S)	10 9	11 9	10 0	3 9	5 2	20 8	1 9	1 6	1 8
1 2	22 4	21 9	22.8	14 0	19 4	28 1	14 0	19 8	30 5
3–4	31 9	29 1	34 6	30 7	32 4	25 9	39 5	37 8	29 2
5–6	21 3	20.9	21 7	28 7	24 1	14 5	30 6	2 4	12 9
7–	11 6	13 8	9 5	20 2	17 2	7 9	12 7	11 3	5
POTATOES PER DAY									
0 POTATO(ES)	1 7	4	2 7		6	7	1	2 2	1 8
1 2	2 3	8 7	4 5	10 1	9 0	7 6	46 0	5	2 6
3–4	3 6	37 2	1.8	46 5	39 5	31 8	3 3	42 8	0 1
5–6	19 3	31 2	8.3	30 0	31 0	32 1	6	6 9	30 1
7–	10 5	20.1	1 5	12 4	18 2	25 6	1 2	1 3	2 8

TABLE 9. ANNOYANCE REACTIONS AT PLACE OF WORK

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-55
TOTAL	32174	15403	16771	3182	166	6921	351	5330	7075
NOISE									
ANNOYED	22 7	34.1	12.1	33 3	3 5	32.0	9	10.6	15.1
VERY ANNOYED	7 2	11 5	3.1	19.9	13.3	10.5	2.4	2 5	3 7
VIBRATIONS									
ANNOYED	8 8	13.3	3.2	13.7	14.1	12.5	2.4	2.5	3 9
VERY ANNOYED	2 0	3		3 5	5 9	3.1	7		8
DUST SOOT SMOKE OR GASES									
ANNOYED	17 4	27 1	9 4	26.2	31 2	24.0	8.1	7 9	11 2
VERY ANNOYED	5 7	9.0	2.4	8.2	11 1	8.0	2 5	2 1	3 9
ODOR ANNOYED	11 2	14.	7 7	14.2	16 2	14.2	4.5	.2	9.6
VERY ANNOYED	2 7	3.0	1.	3.	4.	3.3	1	1.4	2 0
POOR/GLARING LIGHTING									
ANNOYED	10.	12.5	7	13.2	13	11.8	6.3	6 0	9 3
VERY ANNOYED	2	2 7	1 4	2 3	3.7	2 2	1.1	1	1 7
HUMIDITY									
ANNOYED		7 5	8.4	7	8.5	6.6	5 2	5	6 1
VERY ANNOYED	2.4	2 8	1 3	1.8	2.2	1.6	1 1	1 5	1 3
EXTREME TEMPERATURE CHANGES									
ANNOYED	13 6	17 0	9 2	14 3	19 1	13 3	7 3	7 4	11.6
VERY ANNOYED		5 1	2 3	4.	6.2	4	2 1	1 9	2 0
TOO HOT ANNOYED	11	1	1	1 3	16.3	13	8 2	6 1	10
VERY ANNOYED	3.4	4.9	2.	4.0		5	2	2 2	3 7
TOO COLD, ANNOYED	10 1	13.	2	13 7	14.	12 0	5.	5	.6
VERY ANNOYED	6		2.0	4.	4.	3.5	1 9	1	2.7
UNSUITABLE WORK POSTURES									
ANNOYED	14.	9.3	0.7	1 .2	19 6	20.3	8.4	8 9	13.3
VERY ANNOYED	3 8	8.2	2.3	5	5 7	5.0	2.2	2 2	2

INCLUDES VERY ANNOYED

TABLE 10 ANNOYANCE REACTIONS PLACE OF RESIDENCE

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26-35	36-45	46-55
TOTAL	32 74	5603	77	3182	496	6921	351	5330	7075
TRAFFIC NOISE									
ANNOYED	10.2	10.		12 1	10	10.2	1	8.	9.8
VERY ANNOYED	2 0	2 0	1	2	2.1	1.7	2 1	1.6	2.8
AIRCRAFT NOISE									
ANNOYED	5.1	3.	4.7	4.8	9	3.8	9	4 9	4 9
VERY ANNOYED	8	9	7	1 4	3	9			
INDUSTRIAL NOISE									
ANNOYED		2 3	1	2.	2.1	2 3	1 4	1 3	1
VERY ANNOYED					6		3	3	3
NOISE FROM NEIGHBORS									
ANNOYED	.2	8.	10 8	6.	2	6.8	7	8 5	12.5
VERY ANNOYED	1.	1	1.7	1	1	1.4	1	1 3	2.2
ODOR									
ANNOYED	3.7	8	3	7	2	8.0	9.	7	9.
VERY ANNOYED	1 7	1	1.8	1	1 3	1.6	2 0	1 7	1 7
AUTOMOBILE EXHAUST/GASES									
ANNOYED		6.7	9 9		6.0	7 8	3	8	10 2
VERY ANNOYED	2.	1.	2.3	1 7	1 3	1.	2.	1	2 7
AIR POLLUTION									
ANNOYED		8.8		7 9	8.0	6.7	9	5	9
VERY ANNOYED	2 2	2 0	2.	1	2 9	2 1	2.8	1	2 7
DUST SOOT									
ANNOYED	10	8	12.0	8.	9.7	7.8	11 9	12.2	11.
VERY ANNOYED	2.	2.	3.	2.0	2	2.0	1.2	2	1.1
WATER POLLUTION									
ANNOYED	.2	4 1	3		4.1	4.1	1	3.4	3.0
VERY ANNOYED	1 2	1.2	1.2	1	1	1.0	1 1	9	1.6

INCLUDES VERY ANNOYED

TABLE A11 ANNOYANCE REACTIONS GENERAL MEASUREMENTS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26 35	36 45	46-58	26-35	36-45	46-59
TOTAL	32374	15603	16771	3602	4846	6921	4353	5330	7075
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT	31 6	30.0	33.1	29 5	30.7	29 8	32 0	31 1	35 2
VERY ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT	8 2	7 6	8 8	8 0	7 3	7 6	8 7	7 6	9 8
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE 0	68 4	70 0	66 9	70.5	69 3	70 2	68 0	68 9	64 8
1 2	22 6	21 4	23.6	20 9	22 1	21 2	22 4	23 2	24.8
3-4	4 4	4.1	4.6	6 2	6 0	6 0	6 8	5 8	7 2
5-6	2 2	2 1	2 3	1 8	2 1	2 1	2 3	1 8	2 7
7-	3	5	4	5	5	4	5	4	5
ANNOYED AT PLACE OF WORK BY ANY AGENT	42 1	52 8	32 2	51 6	57 1	50 4	27 3	28 1	38 4
VERY ANNOYED AT PLACE OF WORK BY ANY AGENT	15 8	21 6	10.5	21 0	24 7	19 3	8 9	9 2	12 5
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK 0	57 9	47 2	67 8	48 4	42 9	49 6	72 7	71 9	61 6
1 2	21 8	24 2	19 5	23 6	25.7	23 6	16.9	17 3	22 7
3-4	11 5	14 9	6.3	14.9	16.2	1 0	6 9	7 3	10 0
5-6	5 6	8 1	3.2	7 7	8 9	7 8	2	2 5	1
7-8	2 4	4 0	1 0	4.0	4 7	3 5	6	7	1 6
9-	9	1 5	3	1 4	1 7	1 5	3	2	2

TABLE A12 EDUCATION OCCUPATION AND WORK SITUATION

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	6-58	26-35	36-45	6 59
TOTAL	3237	15603	16771	3502	4846	6921	4353	5330	7075
MANDATORY 9 YEARS OF EDUCATION	47 4	46.7	48 0	54 6	43 1	44 9	59 8	44 7	3.2
OTHER EDUCATION	51 7	52 4	51 0	44.4	56 4	54 2	39 3	54 3	55 8
A JOB OTHER THAN THE PRESENT FOR MORE THAN 5 YEARS	25 7	27 2	24 3	40 2	35 2	14 4	33 3	30 8	14.8
PRESENT GAINFUL EMPLOYMENT FULL TIME	51 4	70 4	34 1	84 5	88 6	50 1	31 7	32 0	37 2
PART TIME	17 3	2 5	21 4	1 2	1 9	3 6	34 0	26 9	9 4
NOT GAINFULLY EMPLOYED	32 9	22 5	40.7	9 0	7 3	0 7	30 7	38 1	43 8
TIME FROM HOME TO JOB 15 MIN	3 4	40.3	29 1	48 9	47 6	30.0	37 1	30 6	23 0
16-30	18 5	20.6	16.5	22 8	24 0	15 6	17 8	17 1	15 2
31-60	8 0	9 0	7 1	9	12 1	6.4	6 5	7 5	7 2
61	8	1 1	5	1 1	1 6	7	4	6	6
WORK AT HOME	1	1 2	1 5	1	1 1	9	2 2	2 8	8
TRANSPORTATION TO WORK WALK	13 8	10.7	16 8	1 1	11 4	8.3	22	16 3	13
BICYCLE	11 4	12 4	10 5	17 6	14 8	7 8	15	10 9	7 2
MOTORCYCLE MOTORSCOOTER	1 1	2 1	2	2 1	1 6	2 8	1	2	3
CAR	33 7	48.0	20.5	55.2	63 2	33 3	33 3	2 5	19 7
PUBLIC TRANSPORTATION	22 3	8 1	14 2	7 6	9 3	7 6	13 2	13 8	19 0
CHANGE OF EMPLOYER DURING THE LAST TEN YEARS 0 TIME(S)	37 9	37 9	37 9	47 8	29 8	38 8	48 2	31	35 8
1 2	27 8	27 9	27 8	30 9	37 6	19	25	3 6	22 5
3-4	13 0	14 0	12 1	8	18 8	13.8	4	13 7	15
5-6	3 4	2	2 6	2 2	5 3	.6		2 5	8
7-	1 8	2 7	9	1 1	3.	3 1	2	8	1 5

TABLE 12. EDUCATION, OCCUPATION AND WORK SITUATION CONT'D.

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-55	26-35	36-45	46-55
TOTAL	3237	15603	1 771	3902	4066	4921	4353	5330	7075
OVERTIME, AT PRESENT									
YES	15	23.3	7 2	31 3	33 1	15.4	7	7 4	4.6
HOURS/WEEK	4		4	3			8	3	8
1-5	8	18	3.2	12 5	14 2	7 0	2	3 2	3 3
6-10	.2	2	1.	3	9	4.1	1 5	1 3	1 3
11-	3.4	4.1	7	4.3	3 2	2.2	1 0		
EXTRA JOB NOW OR EARLIER									
YES	1 5	10.9	10.3	1 .6	30 1	10.8	6 7	2	13 7
HOURS/WEEK	7		5		1 1	6	3	8	7
1-5	9	3	3	4.2	7 3	4.1	2.	3	4.
6-10	3.7	3.1	2.4	8.0	5 1	3 1	1	2 1	2.2
11-	3 3	4.2	2.3	3.7	4.2	8	1 2	1 9	1 7
SHIFT-WORK NOW OR EARLIER									
YES	14 9	21 1	9.1	24 8	23.	16.4	9.8	9.6	8.
-5 YEARS	10 2	14.2	6.	11.	16.3	1 3	4 5	6	7 4
6-10	2 1	2 9	1 2	4.3	4.8		2 7	1 7	6
11-15		1	3	3 1	2 8		7	5	
16-	7	1.2	3	4 1	6		9	1	
PIECEWORK NOW OR EARLIER									
YES	2	37	12.3	30 7	5 7	30.2	13 4	13	10.6
-5 YEARS	12 9	10.3	9	10.8	18	22	6.0	5	8.
6-10	8.4	6.7	3	8.	14.3	4.9	3.7	2 8	1 1
11-15	2 3	3		2	7	3	1.3	1 0	
16-	1	3.6		11 7	3 2		1.2	2	
UNEMPLOYED NOW OR EARLIER									
YES	8	11 2	8.6	2	10.	13.8	5	.8	12.5
YEARS	9	7.2	4.8	8	4.4	9.7	2.8	2 5	8.
1	2 4	2	2.2	2 1	2.	2 7	1.	1 9	2.8
2-	5		7			2	1.0		

TABLE 13. RESIDENCE, SATISFACTION, SENSE OF WELL-BEING ETC.

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-55	26-35	36-45	46-55
TOTAL	32374	19603	24772	3902	444	4922	3 3	8330	7879
TYPE OF DWELLING									
APARTMENT	34 1	38.0	8.1	31	9.2	39.5	33.7	37 1	46.4
COOPERATIVE APARTMENT	11 1	10 2	11 8	0	12 5	6	11	11.	10.
TERRACE HOUSE, ROW-STYLED HOUSING	6.3	6.3	6.5		7 0	9	7 3	6 8	3
HOUSE	36	37 5	34.9	8	34 2	34.	40	35	30.5
OTHER		7	5.8	7	5 1	8.5	6.1	3	7.2
NUMBER OF ROOMS									
-2	24	24	8.	1	2 5	27	18.2	28.1	31.4
3-4	44.	44.	7 2	81 9	51	39 2	51 1	3.1	40.4
5-	27 1	7	26.7	29.8	1.	33.4	30.9	26.1	25.1
NUMBER OF PERSONS LIVING IN THE DWELLING									
	3	6	4	8	10.3	8.	5.8	7	
3-	4	4.8	8.1	3	0.	33.	41.4	36.6	1.8
4-5	3	44.2	44.9	3 5	5	44 0	43.1	51.8	39 6
6-		5	6.	6.3	2 8	11 7	6.9	.2	8.1
PETS	37 2	36.1	38.2	1.2	30.1	37	7	34 7	37 5
DWELLING-SATISFACTION									
VERY SATISFIED	52		54	54.2	48.7	4	57	55 6	52.8
RATHER SATISFIED	37	39		35.2	40.8	3 3	34 7	36.4	37.5
NOT ESPECIALLY SATISFIED		1	4.2	.4	7	7	8 3	8	7 3
DISSATISFIED	1	1.	1.	1	1 8	3.0	1 2	1 3	2.2
MUNICIPALITY OF RESIDENCE									
VERY SATISFIED	54.2	54.2	56.0	59 3	53	1 7	61 5	9.6	52.7
RATHER SATISFIED		1.	1.	33.	37	34.3	20 7	30.4	34 6
NOT ESPECIALLY SATISFIED		5	5	5	5	5	3.8	8	8.4
DISSATISFIED	1.4	1 5	1	5	1	2.1		9	2.1
NEIGHBOURHOOD									
VERY SATISFIED	59	57 6	61.7	44.3	54.2	53.5	64.3	63.6	5 5
RATHER SATISFIED	29	31.2	27 7	37	32	32.3	23.4	2 .8	10.6
NOT ESPECIALLY SATISFIED	6.3	5	1	4.	7.4	7 1	3 8	6 2	7.4
DISSATISFIED	1 7	1	1.	5	1 9	2.	9	1.6	2 3

TABLE A11 ANNOYANCE REACTIONS GENERAL MEASUREMENTS

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26 35	36-45	46-58	26-35	36 45	46-55
TOTAL	32374	15603	14771	3802	4846	6921	4353	5330	7075
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT	31 6	30.0	33.1	29 5	30.7	29 8	32 0	31 1	35 2
VERY ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT	8 2	7 6	8 8	8 0	7 3	7 6	8 7	7 6	9 8
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE 0	68 4	70 0	66 9	70 5	69 3	70.2	68 0	68 9	64 8
1 2	22 6	21 4	23.6	20 9	22 1	21 2	22 4	23 2	24.8
3-4	4 4	4.1	4.6	4 2	4 0	4.0	4 8	5 8	7.2
-6	2 2	2 1	2 3	1 8	2 1	2 1	2 3	1 8	2 7
7	5	5	4	5	5	4	5	4	5
ANNOYED AT PLACE OF WORK BY ANY AGENT	42 1	52 8	32.2	51 6	57 1	50 4	27 3	28 1	38 4
VERY ANNOYED AT PLACE OF WORK BY ANY AGENT	15 8	21 4	10.5	21 0	24 7	19 3	8.8	9 2	12 5
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK 0	57 9	47 2	67 8	48 4	42 9	49 6	72 7	71 9	61 6
1 2	21 8	24 2	19 5	23 6	25.7	23 6	16.9	17 3	22.7
3-4	11 5	14 9	8 3	14 9	16.2	1 0	6 5	7 3	10 0
5-6	5 6	8 1	3.2	7 7	8 9	7 8	2.7	2 5	4 1
7-8	2 4	4 0	1.0	4 0	4 7	3 5	6	7	1 4
9-	9	1 5	3	1 4	1 7	1 5	3	2	2

TABLE A12 EDUCATION OCCUPATIO AND WORK SITUATION

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26-35	36-45	46-58	26 35	36-45	46 55
TOTAL	3237	15603	14771	3502	4846	6921	4351	5330	7075
MANDATORY 9 YEARS OF EDUCATION	47 4	46.7	48 0	54 6	43 1	44 9	59 8	44 7	3 2
GHER EDUCAT ON	51 7	52 4	51 0	44.4	56.4	54 2	39 3	54 3	55 8
A JOB OTHER THAN THE PRESENT FOR MORE THAN 5 YEARS	25 7	27 2	24 3	40 2	35 2	14 4	33 3	30 8	14 0
PRESENT GAINFUL EMPLOYMENT FULL TIME	51 6	70 4	34 1	84 5	88.6	50.1	31 7	32 0	37 2
PART TIME	17 3	2 5	21 4	1 2	1 9	3 6	34 0	26 9	9 4
NOT GAINFULLY EMPLOYED	31 9	22 5	40.7	9 0	7 1	0.7	30 7	38 1	48 8
TIME FROM HOME TO JOB 15 IN	34 4	40.1	29 1	48 9	7 6	30 0	37 1	30 6	23 0
16-30	18 5	20 6	16.5	22 8	26 0	15 6	17 8	17 1	13 2
31-60	8 0	9 0	7 1	9 9	12 1	6.4	6 5	7 5	7 2
61	8	1 1	5	1 1	1 6	7	4	6	6
WORK AT HOME	1	1 2	1 5	1	1 1	9	2 2	2 8	8
TRANSPORTATION TO WORK WALK	13 8	10 7	16 8	1 1	11 4	8 3	22	1 3	13 6
B CYCLE	11 4	12 4	10 5	17 6	14 8	7 5	15	10 9	7 2
MOTORCYCLE MOTORSCOOTER	1 1	2 1	2	2 1	1 6	2 3	1	2	3
CAR	33.7	48.0	20.5	55 2	63 2	33 3	23 3	2 5	15 7
PUBLIC TRANSPORTAT ON	11 3	8 1	14.2	7 6	9 3	7 6	13 2	13 8	15 8
CHANGE OF EMPLOYER DURING THE LAST TEN YEARS 0 TIME(S)	37 9	37 9	37 9	47 8	39 8	38 3	48 1	32	35 8
1 2	2 8	27 9	27 8	30 9	37 6	19	25	3 6	32 5
3-4	13 0	14 0	12 1	8	18 8	13 8	4 9	13 7	15
5-6	3 4	2	2 6	2 2	3 3	6		2 5	0
7	1 8	2 7	9	1 1	3.	3.1	2		1 5

TABLE 8 PERSONAL DATA, MEN

	MEN Z OZ			MEN MZ			MEN DZ		
	TOTAL	MZ	DZ	26-35	36-45	46-58	26-35	36-45	46-58
TOTAL	5996	2293	3703	516	718	1058	823	111	1764
TWIN PAIR LIVING TOGETHER	28	30.0	27	4.7	5.2	89 1	2 4	3 8	54.0
TWIN PAIR LIVING SEPARATED	9.1	61.0	73 1	93.2	93.9	3 2	96.6	95.	1 7
AGE AT SEPARATION									
3 YEARS	8	6	9	1		2	1 8	1.	
4-10		8		1 0		1		8	1
11-20	36.6	32.	42.3	5 1	41.8	23 2	56 1	83.6	28.7
21-30	25.	36.	21 7	42 6	44.8	13.4	30 3	36.	11 5
31-	6	1.6		3.7			1 2	3	
CONTACT WITH TWIN PARTNER									
DAILY OR ALMOST DAILY	39 0	6.2	33 2	24.8	32.8	71 9	0	13.2	57.2
ONE OR TWO TIMES/WEEK	23	23 9	22	22 5	39 8	1 7	17	25.7	22 8
ONE OR TWO TIMES/MONTH	21	1 3	24 6	31.2	21.2		35 7	39 1	13.2
MORE SELDOM	13	6.	1 7	1	10.2	1 7	36.0	22	
NEVER		2	3		2		9		2
NUMBER OF SIBLINGS NOT INCLUDING THE TWIN PARTNER									
0 SIBLINGS	13 6	2 1	1 1	17	17 5	19 8	13.7	19.1	13.
1-2		8	44	39.8	7.4	8 0	33 5	7	52
3-4	17	14.0	1	16.3	18.5	1 1	19	17 2	18 7
5-	8.9	8.3	9.2	13	5	9.4	19.6	.6	
MARITAL STATUS TOTAL	5449	2278	367	51	714	13	21	111	178
CONCORDANCE, MARRIED	40.		39.4	7	1 7	12	72 2	1 8	18 1
NOT MARRIED	38 1	9.			12.3	73		.8	1 1
DISCORDANCE	21 5	18.	21 2	16 5	26.	1	23 1	30	1
CHILDREN, TOTAL	5932	226	36 5	512	71	1040	820	1108	1737
CONCORDANCE, YES	3.1	36.	36 2	76 2	53 9	.2	78 7	2 2	5.5
NO	44	5.		6.	19.5	82.3	5 1	13 2	0 9
DISCORDANCE	20	1 5	2		26.	11	2 1	36	3
TWIN CHILDREN, TOTAL	707	190	1 06	44	647	668	777	1008	1119
CONCORDANCE, YES		1			2				
NO	1	7 9	98.2	96.	97.2	99.4	96.	97 7	99 8
DISCORDANCE	1	2.		3.	2.	6	3	2.3	2

TABLE 8A PERSONAL DATA WOMEN

	WOMEN Z			WOMEN			WOMEN Z		
	TOTAL	MZ	DZ	26-35	36-4	46-58	26-35	36-45	46-5
TOTAL	6979	2736	1	1	80	1242	1843	104	1 8
TWIN PAIR LIVING TOGETHER		23.	1 2	2 3	2.	1		7	7
TWIN PAIR LIVING SEPARATED	8.	5.		96.	4.		97 3	4.	9 3
AGE AT SEPARATION									
YEARS			2	1		2	2	8	
4-10		2				1			3
20	58.	0.			1 8	7.2	7	4.5	58.
30	1	2	1 3	56	30.8	11	1	1	.3
31				1	8			2	
CONTACT WITH TWIN PARTNER									
DAILY OR ALMOST DAILY	1	8	33.6	30.	40	72	1 1	9 1	54
ONE OR TWO TIMES/WEEK	3.		31	3.	34.1	8	3	37	37
ONE OR TWO TIMES/MONTH		3.	21	3	13.6	4.0	29	.6	11
MORE SELDOM				6.	4.5	1	20.6	11 0	3 1
NEVER		1		7			9		2
NUMBER OF SIBLINGS NOT INCLUDING THE TWIN PARTNER									
1-2		0.1	5	0	21 0	29.8	1 1	7	5
				2	3	56 2	35 2	44.3	8
3-4	14.	14.		14.	14 5	13	17	17.0	1
5-				4.			1	4	
MARITAL STATUS TOTAL	66	272	46	1	876	1	1062	1246	1 5
CONCORDANCE MARRIED	2.	6.	.3			22		70.9	21
NOT MARRIED	28	1	36.8		.5	59	1	6.	5
DISCORDANCE	3.	4.	25	28.9	32.	1	24.		2 .4
CHILDREN, TOTAL		7722		11	879	1320	1020	1308	1783
CONCORDANCE YES	46	9.7	.8	74	0.3	1	7	4.3	19.
NO	1.	34.	30	6.		71	2	4.	
DISCORDANCE	43		2	1	20	13	2 3	23.2	1 .2
TWIN CHILDREN, TOTAL	97	2322	34 5	79	843	877	1 03	1212	128
CONCORDANCE, YES	1		1	2				2	
NO	97	97	97 1	95.5	47	99.3	94	94.	4.
DISCORDANCE	2	2.7	2	4.3	2.8		5.2	3	

TABLE A13. RESIDENCE SITUATION SENSE OF WELL-BEING ETC. CONT D

	TOTAL			MEN BORN			WOMEN BORN		
	TOTAL	MEN	WOMEN	26 35	36-45	46 58	26 35	36-45	46-58
TOTAL	32374	15603	16771	3802	4866	6921	4353	5338	7073
NEIGHBORS SATISFACTION WITH									
VERY SATISFIED	55 6	53 4	57 7	63.8	54 6	46 8	66 2	60 8	58.1
RATHER SATISFIED	34 6	37 0	32 4	29 4	36.5	41 6	26 0	30 6	37 8
NOT ESPECIALLY SATISFIED	5 1	5 0	5 2	3 2	4 7	6 2	3 2	4 1	7 3
DISSATISFIED	1 5	1 5	1 4	7	1 3	2 2	7	1 8	2 8
TRANSPORTATION FACILITIES									
VERY SATISFIED	37 3	34 6	39 8	40 8	33.2	32 1	44 5	39 0	37 3
RATHER SATISFIED	35 3	37 0	33 7	33 5	38.0	38.3	31 1	34 3	34 9
NOT ESPECIALLY SATISFIED	14 7	15 7	13.7	13 7	17 0	15 9	11 7	14 3	14 5
DISSATISFIED	8 6	9 3	8.0	7 9	8 8	10 4	6 2	7 1	9 7
WORK									
VERY SATISFIED	46.6	44.6	48 5	51 6	49 7	37 1	53 2	51 8	3 8
RATHER SATISFIED	34 2	38 8	29 9	38 1	39 4	38 8	28 0	28 2	32 4
NOT ESPECIALLY SATISFIED	5 9	7 0	4 9	5 0	6.1	8 7	3 5	3 5	6.8
DISSATISFIED	1 9	2 3	1 6	1 1	1 8	3 3	8	8	2 7
WORK ENVIRONMENT									
VERY SATISFIED	39 9	35 7	43 9	42 0	38.3	30 3	48 6	47 5	38.2
RATHER SATISFIED	36 9	41 6	32 6	41 8	42 6	40.7	30 2	30 2	36 8
NOT ESPECIALLY SATISFIED	8 9	11 8	6 2	9 8	12 0	12.8	4 4	5 0	8.1
DISSATISFIED	2 5	3 5	1 5	1.9	3 5	4 3	6	9	2 9
SERVICE ESTABLISHMENTS									
VERY SATISFIED	30 9	28 8	32.8	35.2	29 3	25 1	37 3	33 6	29 5
RATHER SATISFIED	44 8	47 6	42 2	45 4	49 4	47 5	39 3	42 7	3 7
NOT ESPECIALLY SATISFIED	13 4	14 0	12 9	11 9	14 1	15 2	10	11 3	13 9
DISSATISFIED	5 0	5 2	4 7	3 7	4 6	6 6	4 0	4 0	5 7
PARKS AND RECREATIONAL AREAS									
VERY SATISFIED	5 3	55 1	55 6	59 8	55.2	52 5	59 5	57 7	51 5
RATHER SATISFIED	27 8	28 8	26 9	23 4	29 2	28.6	25 3	26 6	28 0
NOT ESPECIALLY SATISFIED	8 8	8 5	9 1	6 2	8 7	9 6	6 9	8 1	11 3
DISSATISFIED	4 3	4 4	4 2	2 4	4 6	5 4	2 4	3 4	6.8

TABLE B 2. MEDICAL DATA, MEN, CONT'D. 2

	MEN Z DZ TOTAL	MZ	DZ	MEN Z 26 35	36-45	5	MEN DZ 26-35	36-45	46-55
SEVERE HEADACHE, TOTAL	5868	2235	3625	506	702	1044	883	109	1726
	1 3	2.1		2 0	2.1	2 1	5	1	5
CONCORDANCE, YES	86.	88.2	86 1	8	86.8	0 5	8 2	82	8
NO	11 8	9 7	13 1	12 6	11 1	7	19.3	1 3	10.1
DISCORDANCE									
HEADACHE VISUAL DISTURBANCES/VOMITING, TOTAL	5811	2230	35 1	580	695	1034	96	1881	1704
	2		1	1 8	3	1		2	
CONCORDANCE, YES	95 5	95.7	95	93.	94 5	7 5	93 8	94.2	7
NO	4.3	4.	5	5 6	3 2	2	7 0	5	2
DISCORDANCE									
IMPAIRED HEARING, TOTAL	5804	2213	359	49	699	101	8C	1079	1710
	2.6	3.1	2 3	6.8	3.0	1 3	3 3	1	1 3
CONCORDANCE YES	83.2	9.1	82 0	7 7	8 8	0	87 1	82	7 8
NO	14.3	11 8	13 8	18	12 2	9 3		19 1	10.9
DISCORDANCE									
ALLERGIES TOTAL	5785	221	3564	93	696	1029	794	107	1698
	5 2	7 2	0	3	5 7	5	2	5	
CONCORDANCE, YES	74.	74.9	73 2	79 5	73.	76	76	8	71
NO	20.1	15.	2 8	16.2	18 8	13 7	21 0	22	23 7
DISCORDANCE									
COLDS, TOTAL	5663	21 5	3488	84	683	1007	75	1044	1644
	8	5.	3 3	6	1 8	3	7	1 0	6.
CONCORDANCE 3- PER YEAR	79	8.8	77	90 9	83 9	72	90 0	82 5	9 3
0-2	17 1	14.2	1 9	9	12.3	1 .2		1	2 7
DISCORDANCE	5	4.	5	2 3	2 8	7.2	9		8 9
DISCORDANCE, 3/2									
SERIOUS ILLNESS TOTAL	5858	2233	341	902	702	10 8	889	1092	1716
	3.9	4.	3 3		4.8	.4	8 2	3 2	2 5
CONCORDANCE YES	3.2	4.	72	62 9	72 5	81 2	1 6	69	79 3
NO	22	20.8	24.2	29.3	2 .6	15	33.3	27 8	18 2
DISCORDANCE									
SICK LEAVE FOR OVER 3 CONSECUTIVE MONTHS ANY TIME, TOTAL	5922	226	3653	909	710	1040	23	1104	1736
	2.0	2.	1	5	3.2	1 8	3 8	2 1	3
CONCORDANCE, YES	81	1	1 1	69	76.8	1	68 1	75 0	91 8
NO	16	15.	1 3	26.1	20.1	5	28.8	22	8 6
DISCORDANCE									

TABLE 82

MEDICAL DATA, MEN

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 26-35	MEN MZ 36-45	MEN MZ 46 58	MEN DZ 26 35	MEN DZ 36-45	MEN DZ 46-58
CHEST PAIN TOTAL	5892	2257	3635	506	710	1040	807	1109	1173
CONCORDANCE YES	10 5	12 8	9 0	14.0	12 1	12 6	11 8	9 7	7 5
No	58 1	59 0	57 5	52 6	56 3	63 9	51 1	55.0	62.1
DISCORDANCE	31 5	28.2	33 5	33 4	31.5	23 5	37 2	35 7	31.4
REPEATED CHEST PAIN TOTAL	5864	2242	3622	505	705	1031	804	1099	1717
CONCORDANCE YES	2 7	4 1	1 9	4 8	3 8	3 8	3 6	1 9	1 1
No	78 8	79 6	78 3	76.0	78 0	82 5	71 7	76.3	82.4
DISCORDANCE	18 5	16 3	19 8	19 2	18 2	13 7	24 7	21 7	16.3
ANGINA PECTORIS EMOTIONAL OR EFFORT TOTAL	5996	2293	3703	516	718	1058	823	1116	1764
CONCORDANCE YES	4	7	2	1 0	1 0	4	4	1	1
NO	94 6	94 3	94 8	91 1	92 9	96 8	91 9	93.8	96.0
DISCORDANCE	5 1	4.0	5 1	7 9	6.1	2 8	7 8	6 3	2 9
SEVERE CHEST PAIN LASTING FOR OVER 30 MIN TOT	5713	2199	3514	497	690	1011	792	103	1611
CONCORDANCE YES	4	4	3	6	4	7	4	5	1
NO	93 2	93 7	92 8	92 0	93 3	94 8	91 5	92.0	94.9
DISCORDANCE	6 4	5 7	4 9	7 4	6 2	4 5	8 1	7 5	5 9

TABLE 82. MEDICAL DATA, MEN CONT D I

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 26-35	MEN MZ 36-45	MEN MZ 46 58	MEN DZ 26-35	MEN DZ 36-45	MEN DZ 46-58
COUGH REGULARLY TOTAL	5831	2235	3596	507	698	1029	791	1091	1714
CONCORDANCE YES	1 0	1 3	8	1 2	6	1 7	5	7	1 8
No	89 7	90 0	89 5	88 2	92 4	89 3	86 7	91 4	89.5
D SCORDANCE	9 3	8.7	9 7	10 7	7 0	8 9	12 8	7 9	9 5
REGULAR COUGH MORE THAN 3 MONTHS/YEA TOT L	5797	2222	3575	501	697	1023	783	1046	1784
CONCORDANCE YES	1	1		4					2
No	97 7	97 7	97 7	97 0	98 1	97 8	95 7	97 4	98 7
DISCORDANCE	2.3	2 2	2 3	2 6	1 9	2 2	4 3	2 6	1.3
PHLEGM MORE THAN 3 MONTHS/YEAR, TOTAL	5728	2193	3535	4 5	691	1034	770	1078	1687
CONCORDANCE YES									
NO	98 2	98.4	98 2	97	98 4	98 9	96 2	98.2	98 8
D SCORDANCE	1 6	1 6	1 8	2 4	1 6	1 2	3 8	1 8	1 6
BREATHLESSNESS TOT L	5780	2224	3556	505	97	1021	800	1059	1690
CONCORDANCE YES	2 3	3.0	1 9	2	2 4	3	2 8	1 6	1 8
NO	82 0	83 7	81 0	79 6	82 2	8 9	76 4	81 3	83 9
D SCORDANCE	15 6	13.3	17 1	17 8	15	9 7	20 9	17 1	15 3
DISCOMFORT AINS ACHES I THE D APHRAGM TOTAL	5710	2141	3519	4 1	691	1009	791	1057	1681
CONCORDANCE YES	4	5 5	3 7	6 5	7 2	3 9	5 2	5	2 8
NO	73 1	74.2	72 5	67 0	67 6	82 1	66 5	64	80
DISCORDANCE	22 5	20	23 8	2 5	25 2	1 1	28 3	30 2	17 7
PAI THE BACK OTAL	57 3	2219	3544	502	496	1020	798	1051	1695
CONCORDANCE YES	4 6	5.4	4 1	7 8	9	6	7 9	3 9	2 5
NO	72 2	75 0	70	6 1	73 1	81 1	57	7 2	78 6
DISCORDANCE	23 2	19 6	25 5	27 1	23 0	1 3	34 7	28	18 9
P IN I THE BACK SHOULDERS OR BACK OF THE NECK TOT L	5763	2214	3544	502	496	1020	798	1051	1695
CONCORDANCE YES	6 0	8	5 5		3	9 8	11	5	2 7
NO	68.5	71 1	66 9	59	67 7	79 1	53.6	62	73 9
DISCORDANCE	25 5	22 1	27 6	31 1	26 0	19 1	35 0	32 8	21

TABLE 82. MEDICAL DATA, WOMEN, CONT'D. 2

	WOMEN TOTAL	% MZ	% DZ	WOMEN 26-35	36-45	46-58	WOMEN % 26-35	36-45	46-58
SEVERE HEADACHE, TOTAL CONCORDANCE, YES NO DISCORDANCE	7 7 3 76 20 1	268 4.5 4.8 16.7	343 3 1 74 22 4	597 7 74.8 20	85 9 9 77 2 17 3	1225 3 82 8 1 7	1027 71 0 25 0	127 1.1 2 8	1757 2 4 7 1 1 2
HEADACHE VISUAL DISTURBANCES/VOMITING TOTAL CONCORDANCE, YES NO DISCORDANCE	6692 1.2 88 1 10.	2 96 1. 40.1 4.	1996 1 0 8 12 2	9 1 2 2 6 1 12	846 2 0 88.8 2	121 1 8 3 7	1312 1 1 82 1 16 8	1251 1 9 1 7	1730 1 8 8
IMPAIRED HEARING, TOTAL CONCORDANCE, YES NO DISCORDANCE	91 8 0	2 1 8 1 7	4017 91 2 8	5 1 5 9 3 10 2	852 91. 6.0	1295 2	1021 4 12 8	1269 2 4 6.	1735 3 92 3
ALLERGIES, TOTAL CONCORDANCE, YES NO DISCORDANCE	6323 7 3 9.7 23 3	2429 1 8 19.	3994 5 9 68 25 7	544 0 71 7 1	846 7 70.1 22 2	1195 10 71 1	1013 5 0 2 .6	12 5 2 75 8	1 71 8 5 49.2 2
COLDS, TOTAL CONCORDANCE 3- PER YEAR 0-2 DISCORDANCE DISCORDANCE, 3/2	54 5 8 19 8 6.8	25 0 5 6.3 3 4.	3959 73 5 2. 2 4	857 1 1 89 3 2 3	838 3 9 8C.3 15 5 8	11 7 11 2 7	1 7 8 9 1 2 3	1239 1 23.7	17 7 44.2 26. 0.2
SERIOUS ILLNESS, TOTAL CONCORDANCE, YES NO DISCORDANCE	792 3.9 7 21 0	1463 5.1 4.1 8.8	40 3 1 74 5 22	3 9. 1 2 9	853 4.6 26.9	121 1 12 5	103 9 64 35 8	2 2. 75.2 22	1 62 2 17
SICK LEAVE FOR OVER 3 CONSECUTIVE MONTHS ANY TIME, TOTAL CONCORDANCE YES NO DISCORDANCE	6851 1 84 2 14.8	7706 2 6 84.9 12.5	125 1 3 3 15 8	609 5. 75 23.2	849 2 82 15 1	1225	104 3 0 71 3 25	129 1 2 81 1	1781 3 2 1

TABLE 82

MEDICAL DATA, WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 26-35	WOMEN MZ 36-45	WOMEN MZ 46-54	WOMEN DZ 26-35	WOMEN DZ 36-45	WOMEN DZ 46-54
CHEST PAIN TOTAL	47 7	2672	4085	598	855	1216	1038	1203	174
CONCORDANCE YES	11 3	12.5	10 8	12 2	11 0	13 7	9 2	10.	11 3
NO	5 6	61 3	58 5	59.	58 0	64 6	57 7		3.8
DISCORDANCE	[illegible] 1	26 2	31 1	28 9	31 3	21 5	33 1	3 8	27
REPEATED CHEST PAIN TOTAL	4725	2 59	4366	597	851	1 09	1032	127	175
CONCORDANCE YES	3 4	1	3 0	4 0	4 1	4 1	3 3	3.6	7
NO	79 4	80.7	78 5	75.0	79.0	84 6	74 7	77 1	81 1
DISCORDANCE	17 2	15 3	18 5	20 9	16.9	11 3	22 0	19 3	15.
ANGINA PECTORIS EMOTIONAL OR EFFORT TOTAL	4909	2736	4173	611	880	1242	1063	1304	1805
CONCORDANCE YES	4	6	3	7	0	5	4	2	2
NO	94 0	94 4	93 8	92 6	93 3	96 0	93 8	2	94.4
DISCORDANCE	5 6	5 0	5 9	4.7	5 9	3 5	.8	1	5.2
SEVERE CHEST PAIN LASTING FOR OVER 30 MIN TOTAL	4623	2639	3984	587	849	1200	1008	1230	1773
CONCORDANCE YES	2	3	2	5	4	3	3		2
NO	94 2	94 7	93 9	94 7	93 5	95	92 8	3.2	93 9
DI CORDANCE	5 6	5 0	5 9	4 8	6.2	4 3	6 9	6 6	5

TABLE 82 MEDICAL DATA, WOMEN CONT'D I

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 26-35	WOMEN MZ 36-45	WOMEN MZ 46-54	WOMEN DZ 26-35	WOMEN DZ 36-45	WOMEN DZ 46-54
COUGH REGULARLY TOTAL	4709	2659	4050	3	851	1218	1033	124	1749
CONCORDANCE YES	1 3	1 5	1	1 0	1 2	2	1 1	6	1
NO	89 9	89 7	8 3	0 7	90 5	49 7	88	6 9	87
DISCORDANCE	9	8 7	10 6	8 3	8 3	9 2	10 6	10 1	11 8
COUGH REGULARLY MORE THAN 3 MONTHS/YEAR, TOTAL	4477	2450	4027	593	8 7	120	1023	1257	1744
CONCORDANCE YES	1	1	1			2	1		1
NO	98 2	99 4	99 1	97 6	98 6	98	7	98 2	8
DISCORDANCE	1 7	1 5	1 9	2 4	1	1	2 5	1 8	1 5
PHLEGM MORE THAN 3 MONTHS/YEAR, TOTAL	49 7	2 18	3959	546	839	1190	1035	123	1717
CONCORDANCE YES			1			1			1
NO	98 8	9 9	98 7	9 5	99 2	0	98	5	1
DISCORDANCE	1 1	1 0	1 2	1 5	8		1	7 5	8
BREATHLESSNESS TOTAL	4473	245	4019	5 2	85	120	10 5	12 5	1737
CONCORDANCE YES		10 9	8	10 8	11 0	10	8 8		9 3
NO	45 2	4 7	2 9	64 2	67 1	2 1	57 5	64	
DISCORDANCE	25	70 4	2 8	25 0	21 9	1 1	35 8	28	26 1
DISCOMFORT PAINS ACHES N THE DIAPHRAGM, TOTAL	4606	30	37 6	5 1	851	11 5	1031	12	1 21
CONCORDANCE YES	5	9	9		11 5	5		8 7	3
NO	63 7	65.0	6 8	5	55 6		59	8	7.2
DISCORDANCE	26 1	5 1	30	25	2 9	21	3 0	2	27.2
PAIN IN THE BACK TOT L	45	2 49	401	89	5	11	102	1256	1734
CONCORDANCE YES	3 3	1	2 8	5	1	2	7	2 5	1 8
NO	78.5	82.	77 0	73 3	7	2	7 3	77	62
DISCORDANCE	18 2	19 1	20 2	1 7	1 1		28 1	20 0	15 7
PAIN IN THE BACK SHOULDERS OR BACK OF THE NECK, TOTAL	66 8	2 9	31	89	59	11 4	132	125	1734
CONCORDANCE YES	5 0	3	2	11 8	5	3	7 8	3.	2
NO	73 5	5.9	73 8	7 9	73 1	91	9	73 7	79 1
DISCORDANCE	21 5	17 8	2 0	21 1	20	1	32 6	7 4	18 7

TABLE 82. MEDICAL DATA, WOMEN, CONT'D. 2

	WOMEN			WOMEN			WOMEN		
	TOTAL	MZ	DZ	24-35	36-45	46-5	24-35	36-4	46-54
[illegible] HEADACHE, TOTAL	7	348	343	997	830	1223	1027	127	1753
CONCORDANCE, YES	3 7	4.5	3 1	4.7	5 5	3	4 0	3.1	2 6
NO	2	4.	76	4.5	17 2	42	71 0	72 8	7 1
DISCORDANCE	20 1	14.7	22	20 8	17 3	14 2	2 0	2	1
HEADACHE [illegible] DISTURBANCES/[illegible]									
TOTAL	6632	2 6	3 9	3 1	846	121	1312	1252	1730
CONCORDANCE, YES	1 2	1	1 6	2 2	2	1	2 1	1	
NO	8 1	48.1	8	4 1	62.2	3	2 1	44	1 6
DISCORDANCE	18.7	4.	12 2	12 7	2		1 6	13 7	8
[illegible] HEARING, TOTAL	46	2 51	4027	1	852	1205	1921	1269	1735
CONCORDANCE, YES		8		1 9					
NO	91	1.3	91 2	3	3	92	97	2	2
DISCORDANCE	8 0			1 2	.0		12 0		
ALLERGIES, TOTAL	6623	2429	3944	396	844	1194	1 1	1246	1 11
CONCORDANCE, YES	7 3	3		0	7 7		5 0		3 9
NO	4.	1.4	68	71	0.1	71		2	2
DISCORDANCE	2	19.7	25	1	22.2	17	25	8	2 5
COLDS, TOTAL	54	2998	3939	357	38	11 2	1	23	1 7
CONCORDANCE 3- PER YEAR	9	8		1 1	3 9	12	7	2 1	8 9
0-2	4.3	6.	73 5	89	8C.	7 2	8 5	1	64.7
DISCORDANCE	1	5	2. 2		18 8		11	3.7	26.
DISCORDANCE, 3/2	4.	4.		3	3.8	7	2 3	5	1 2
[illegible] ILLNESS, TOTAL									
CONCORDANCE, YES	792	34 3	68	4	33	121	2 37	1 87	1 2
NO		8 1	3 1		4.4			8	2
DISCORDANCE	7 1	4.	74	3.2	7	8	64	.2	7
	21 8		22	2 5	26.	12	33 5	22 2	17
SICK LEAVE FOR OVER 3 CONSECUTIVE [illegible]									
ANY TIME, TOTAL									
CONCORDANCE YES	831	7706	123	609	849	1229	046	295	17 1
NO	1	2	1 3	9.	2		3 0	1.2	
DISCORDANCE	2	84.9	83	78.	62 2	9.	1 3	9	2.4
	14.	12.	15	23.2	19 1		2 7	2 1	7 1

TABLE 83 PHYSICAL ACTIVITY AND HEIGHT AND WEIGHT MEN

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 24-35	MEN MZ 36-45	MEN MZ 45-58	MEN DZ 24-35	MEN DZ 36-45	MEN DZ 46-58
PHYSICAL ACTIVITY AT WORK, TOTAL	5739	2211	3528	501	701	1059	795	1070	1655
CONCORDANCE LOW GRADE 1 2	36 3	40 5	33 6	37 5	38 2	43 0	31 7	30.0	36.9
HIGH 3-4	32 0	34 0	30 7	30 3	34 7	35 4	28 2	30 9	31.8
DISCORDANCE	31 7	25 5	35 7	32 1	26 1	21 6	40.1	39 1	31 1
DISCORDANCE 2/3	11 2	9 7	12 1	12 0	10 4	7 9	15 9	15 6	9
PHYSICAL ACTIVITY DURING LEISURE TIME, TOTAL	5921	2260	3661	510	708	1041	809	1106	1746
CONCORDANCE LOW GRADE 1-4	45 0	7 1	43 6	52 5	48 7	43 4	49 6	6.6	38.9
HIGH 5-7	23 6	28.2	20 7	17 3	23 7	36 7	11 5	1 5	39 5
DISCORDANCE	31 5	24 6	35 7	30 2	27 5	19 9	38 9	38 4	32
DISCORDANCE 4/5	10 5	9 7	11 0	12 5	12 1	5 4	16 6	9	9.4
OVERWEIGHT TOTAL	5826	2219	3607	502	699	1017	802	1092	171
CONCORDANCE YES (1 20)	2 3	3.1	1 8	6.0	3 6	1	5 2	2 1	1
NO	87 7	89 3	86 7	76 9	88.0	96 4	71 1	85 0	93.0
DISCORDANCE	10 0	7 6	11 5	17 1	8 4	2 4	23 7	12 9	4.9

TABLE 83 PHYSICAL ACTIVITY AND HEIGHT AND WEIGHT WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 26-35	WOMEN MZ 36-45	WOMEN MZ 46 59	WOMEN DZ 26 35	WOMEN DZ 36-45	WOMEN DZ 46-59
PHYSICAL ACTIVITY AT WORK, TOTAL	6452	2 93	3879	570	832	11 9	893	1227	1697
CONCORDANCE LOW GRADE 1 2	41 0	4 0	37	35	38 9	34	2 0	29 1	9 1
OH 3-4	23 4	2 2	22 9	24 7	29 6	1 3	27 0	2 0	1 1
DISCORDANCE	35 6	29 8	39 5	37 9	3 5	22 5	3	44 9	32.8
DISCORDANCE 2/3	10.3	1 5	20 1	22 3	18 6		45 9	29	12 3
PHYSICAL ACTIVITY DURING LEISURE TIME TOTAL	4011	2	19	600	5.9	12	10 6	17 3	1713
CONCORDANCE LOW GRADE 1-4	60	42 0	63 0	39 7	6 3	40	5 9	42 7	9 1
HIGH 5-7	13 9	17 1	1 2	11 8	1 6	12	1		15.1
DISCORDANCE	25 7	20.9	29 8	28 3	21 4	16	31 9	28	29 9
DISCORDANCE /5	11 5	10	12 1	1 2	11 1	9 3	13 9	11 6	11
OVERWEIGHT TOTAL	730	2471	4059	5 2	8 1	1219	1032	127	17 3
CONCORDANCE YES (>1 20)	7	3	2 3	9 4	2		9	1	7
NO	88 9	1 2	87 3	74 7	91	97 0	70 3	8	9 3
DISCORDANCE	8.3	5 6	10	11 7	5.8	2 5	22 8	9	3 7

TABLE 94. SMOKING HABITS MEN

	MEN			MEN			MEN		
	N Z D Z			N Z			D Z		
	TOTAL	NZ	DZ	26–35	36–45	46–55	26–35	36–45	46–55
SMOKING EXPERIENCE, TOTAL	5940	2275	3665	913	714	1947	1	1111	173
CONC. NONSMOKERS	27 6	3 7	23.8	26.7	27 9	1 1	18 9	19.5	29.5
SMOKERS	90 6	52.1		84 4	57 8		52 8	52 8	
PRESENT SMOKERS	34 8	34.0	32 8	32	39.	34	39	32 8	33
FORMER SMOKERS	3.	4.3	8		4.5	3 3	5	3 9	2 2
DISC. 5 YRS, NS	21 3	14.9	26 2	19.7	14.0	11	27 9	29	23
PRESENT 5 YRS, NS	15 7	4	19	11 3	9.9	8 1	1 3	21 5	1
FORMER 5 YRS, NS	5.8	4.	6	7	4.1	3 5	7		
PRESENT 5 YRS, FORMER S	12 8	11 8	13 9	19 0	13	2	1	14.8	18.2
CIGARETTES TOTAL	5910	2267	364	510	709	1947	04	1104	1733
CONC. CIGARETTES	40 7	1	43 1	1	8	37 2	41 2	44.2	37 8
NOT	34.0	40.3	30 1	33 3	32.		26	23.8	4.8
DISC. CIG YRS NOT CIG	24.5	17 1	29	28.	17	13 3	33 3	31 5	25
CIG YRS, NS	17	11 2	22	16 1	11	5	2 5	24.	1
CIGARETTES, PRESENT									
CONC., CIG PRESENT	1	21 0	17	13.	21 7	23 2	15 0	1 8	1 2
NOT CIG	51 2	54.2	48 1	55 5	50.9	60 2	49	43 8	50
DISC. CIG PRESENT YRS NOT CIG PRESENT	29 1	22.	33.6	27	26.4	1 1	1 7	38 7	29 8
CIG PRESENT YRS, NS	10.8	4.1	13 3	8 2	6.3	4	1 1	15 1	12 2
CIGARETTES QUANTITY									
1 DISC., 5 CIG	9 7	8 3	18	6 8	1 8	4	13 1	1 7	8.1
10 CIG	2.7	1.	2	2.4	2 5	1.1		.4	1
2 DISC., 5 CIG	3 7	3.	3 8	2 4	3.1	2	2	5 8	2
10 CIG			1 2	2	7	7	1 9	1.7	5
FILTER-CIGARETTES									
TOTAL	2 51	94	2580	21	344	52	62	594	437
CONC. WITH	39 3	2.	7.0	32	6.	64	25	94 8	7
WITHOUT	0		8	23.3	9.2	2	1 3	7 9	3 8
WITH WITHOUT	2		1			1			3
DC. WITH YRS. SHORT	21 9	1 7	23 3	30.	26.3	7	3 8	26.6	12 9

PRESENT AND FORMER SMOKERS
2) PRESENT SMOKING

TABLE 94. SMOKING HABITS MEN CONT D.

	MEN			MEN			MEN		
	Z						D Z		
	TOTAL	NZ	DZ	26–	36–45	46–	26–35	36–45	46–
CIGAR/CIGAR-CIGARETTES	5794	2226	3568	99	96	1032	768	1075	1703
CONC. CIGAR/CIGAR-CIG	4.3	5.3	7	5	.8	8 3		3 3	3
NOT	7 6	76.	7	72 5	5	90 1	71 7	72 2	77
DISC. CIGAR/CIGAR-CIG YRS, NOT	19.8	1	21	21	1 .6	1 .4	2 0	2 8	18 5
CIGAR/CIGAR-CIG YRS NS	4.5	3.	5	4.3	2.3	2	5 8	6 1	.6
CIGAR/CIGAR-CIG PRESENT									
CONC. PRESENT	2	2.	1 7		2.3	8	1.8	1 5	1 8
NOT PRESENT	8 3	84.6	85 1	82	83 1	8	83	83 2	85.6
DISC. PRESENT YRS, NOT PRESENT	3.	12.	13	5	1 3	10 3	1 5	15.8	12 3
PRESENT YRS, NS	2		3	8	1.	1	3	4.0	2.6
PIPE, TOTAL	5 80	231	3577	490	697	1 5	789	1079	1709
CONC. PIPE	1	1 5	1	1 7	2 2	.6	18	18 7	13.0
NOT PIPE	5	3	50 8	91 8	3.	3 5	3 1	44.	36 8
DISC. PIPE YRS, NOT PIPE	7	20.7	32 1	3 5	23 1	16.5	36 1	36 0	27 7
PIPE YRS NS	9 5		12.0	7	6.2	8	13	1 .6	7
PIPE, PRESENT									
CONC. PIPE PRESENT		3	6	10.2	9.9				
NOT PIPE, PRESENT	3		68 5	69	7 .2	6.	65 4	64.	72 1
DISC. PIPE PRESENT YRS, NOT PIPE PRESENT		16.	2 8	1	1 .2	1 8	26 4	28 3	22 8
PIPE PRESENT YRS, NS	3	3.1	7	3.0	3.6	2 4	7	2	3.7
SNUFF/CHEWING TOBACCO, TOTAL	5772	2217	35	1	2	10 3	789	1068	1 93
CONC. SNUFF/CHEW TOB.	8	6.		5	5.	12	3 5	1	7
NOT SNUFF/CHEW TOB.	2		78.8	3	6.	71	3	8.4	9 8
DC. SNUFF/CHEW. TOB. YRS. NOT SNUFF/CHEW TOB.		13.	2		1 8	13	12.8	22.8	26.9
SNUFF/CHEW. TOB. YRS, NS		3.		0	2	4.5	3.	4.	7
SNUFF/CHEW TOB PRESENT									
CONC. PRESENT	5			4.	7 5	9 2	2	.6	5 8
NOT PRESENT	8 8	8	76 5	7 2	79.	77	.4	7	72 7
DISC. PRESENT YRS, NOT PRESENT	1	11.	10	8	17	13	11 5	1 .8	2 1
PRESENT YRS, NS		5		1.	2 2	2	2.7		
TYPE OF EXPERIENCE, TOTAL	5996	293	3703	51	71	1095	23	1116	1764
ONL. CIG YRS NS		4.		4.		3 7	2	8.1	4.8
ONL. CIGARS YRS, NS	5			1	3	8			3
ONLY PIPE YRS, NS				2		2	1 3		6
ONL. SNUFF YRS, NS	8	3		6		8	7	3	3 1

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
SMOKING DISCORDANCE TOTAL	6 84	5 5	40 9	401	873	1218	1329	1290	1727
CONC. NONSMOKERS	38 2	49 4	34 7	48	36 1	4 8	41 5	3.4	31.
SMOKERS	3 6	18.7	6 9	28 8	42 1	40	2 9	4.1	43.3
PRESENT SMOKERS	24 0	25 9	2 8	14.8	29 4	27 9	12 3	22 5	29 1
FORMER SMOKERS	3 6	4 3	3	0	5 0	3 9	1 9	0	
DISC 5 VRS NS	23 7		7 8	21 8	20	13 1	34 7	29	22 3
PRESENT 5 VRS NS	16 4	10	20 1	13 6	11 8	8 9	26 0	19 6	16 6
FORMER 5 VRS NS	7 2	4.4	7 8	8 2	8	2	8 6	10 3	9
PRESENT 5 VRS FORMER S	9 9	9 5	10 9	8 0	8 6	8 7	9 7	12 6	13 6
CIGARETTES TOTAL	6340	711	4129	605	877	1 26	1343	1299	1714
CONC. CIGARETTES	36 8	39 3	35 8	28 6	42 4	40 1	21	8	1.2
NOT	38	4 1	35 5	49 8	36 5	44 7	42 5	31 1	34.5
DISC. CGT VRS NOT CGT	24 0	17 3	28 3	21 3	20 8	12 9	3 1	2 3	22 9
CGT VRS NS	23 1	16 8	27 2	21 2	20.0	12 4	33 8	29 3	21 6
CIGARETTES PRESENT									
CONC CGT PRESENT	23 4	15 5	22 0	14.5	28 7	27 7	11 6	27 8	29 3
NOT CGT	50 0	4 9	44 8	41 7	50 4	54 6	51 2	9 6	3.9
DISC CGT PRESENT VRS NOT CGT PRESENT	26 2	13.3	30 8	21 5	20 5	17 3	34 6	12.2	27 5
CGT PRESENT VRS NS	15	10.5	19 4	13 2	11 6	8 4	25 3	19 1	16.2
CIGARETTES QUANTITY									
1)DISC. 5 CGT	5	3 8	6 6	4 8	4 6	2 9	4 5	8 8	6.8
>10 CGT	1 1	6	1 3	8	4	6	1 0	1	1 1
2)DISC. 5 CGT	3 0	2 1	3 6	2.1	2 5	1 8	2 1	4 1	4.2
>10 CGT	5	4	5	7	3	2	3	8	3
FILTER-CIGARETTES TOTAL	2521	1020	1501	174	370	76	293	509	739
CONC. WITH	8 4	5 7	83 9	47 2	85 1	97 3	55 7	0.4	85 9
WITHOUT	1 7	1 4	1 8	3 4	2	2	5 9	2	
WITH WITHOUT									
DISC. WITH VRS WITHOUT	9 7	8 9	10 2	23 6	10 5	2 3	22 9	13 4	3 7

1) PRESENT AND FORMER SMOKERS
2) PRESENT SMOKERS

TABLE 84 SMOKING HABITS WOMEN CONT D.

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
CIGARS/CIGAR-CIGARETTES	634	254	3882	568	817	11	963	1202	1454
CONC. CIGAR/CIGAR-CGT		4	2	2		4	2	7	2
NOT	9 3	94 9	93 9	94 0	53 8	96 0	94 6	2	91 7
DISC. CIGAR/CIGAR CGT VRS NOT	2	4 4	5 7	5 3	5	3 2	3 1	5 5	5.2
CIGAR/CIGAR-CGT VRS NS	1 8	1 4	2 0	2 8	1 2	9	2 9	1	1 1
CIGAR/CIGAR-CGT PRESENT									
CONC PRESENT	7	3	1		4			7	1
NOT PRESENT	95	5 0	93 3	95 6	55 0	96	96 3	5 3	9 3
DISC PRESENT VRS NOT PRESENT	3	3.4	4 3	3	4	2	3 6	2	4.8
PRESENT VRS NS	1 4	1 1	1 6	2 1	1 0	4	1 7	1	1
PIPE TOTAL	6344	931	3813	943	813	1152	9 9	11 9	1 53
CONC. PIPE	3		2					2	
NOT PIPE	9	4	97 6	99 6	58 9	97	99 3	98 2	96 1
DISC PIPE VRS NOT PIPE	1	1 3	2 1		1 1	1	7	1	3
PIPE VRS NS	4	2	6		2	2	3	3	8
PIPE PRESENT									
CONC PIPE PRESENT	1	1	1			3		1	2
NOT PIPE, PRESENT	8	2	98 3	99 8	85	99	99	8 7	7 3
DISC. PIPE PRESENT VRS NOT PIPE PRESENT	1	7	1	2		1 3	6	1 1	2.2
PIPE PRESENT VRS NS	3	1	5		1	1	3	3	7
SNUFF/CHEW TOBACCO TOTAL	43 1	2532	3309	5 3	813	1153	9 9	11	1 53
CONC. SNUFF/CHEW TOB		1							
NOT SNUFF/CHEW TOB	9		99	99 6	99	99	9 9	100 0	99 5
DISC. SNUFF/CHEW TOB VRS NOT SNUFF/CHEW TOB	5		5		1		1		1 2
SNUFF/CHEW TOB VRS NS		1		2		1			3
SNUFF/CHEW TOB PRESENT									
CONC. PRESENT		1							
NOT PRESENT	99 6		99	99	9	99	9	100 0	9 .2
DISC PRESENT VRS NOT PRESENT	3	3			1		1		8
PRESENT VRS NS				2					
TYPE OF DISCORDANCE TOTAL	09	2	173	11	893	12	10 3	334	1603
ONLY CGT VRS NS	17 8	1 8	23 1	17 7	17 7	11	28	4 5	19.2
ONLY CIGARS VRS NS	3	3							3
ONLY PIPE VRS NS									1
ONLY SNUFF VRS									

TABLE 85 USE OF PHARMACEUTICALS MEN

	MEN			MEN			MEN		
	TOTAL	NC	CZ	26-35	36-45	46-58	26-35	36-45	46-58
REGULAR INTAKE OF THE PHARMACEUTICAL									
TOTAL	840.	2192	3452	84	679		7	1049	1 34
CONC. YES	1	2.	1 3	3 1	1.6	1 7	2	1 3	6
NO	82	8 7	81.8	83 8	8.	3 9	6.	82.2	84 1
NO RESPONSE	1	14.2	1	16.1	15.	12	20	1 9	13 1
RESTORATIVES									
TOTAL	9446	2116	3353	441	44		73	4	2 1
CONC. REGULAR	1.4		1 1	7		3 9	1	3	2
NOT	0.8	90.	90 3	92 8	93.9	8 7	91 2	1 2	.4
NO RESPONSE	8.1	3		4.3	8.7	4 9	8 7	5	8.
PRESCRIPTION-FREE ANALGESICS									
TOTAL	15	237	3243	7	53	1	765	79	1 59
CONC. REGULAR	1	1	1	2			1	1	1
NOT	8.5	8.	3	95.	8.0	99	8	98 1	7
NO RESPONSE	1	1	1.4	1 8	1 8		.8	1 8	1.3
SLEEPING PILLS TOTAL	9353	198	9087	1	613	19	75	17	1 5
CONC. REGULAR							1		
NOT	98.	9.	8	97 7	8.7	1	94	98 5	99.8
NO RESPONSE	1	1.	1 5	2.3	1.3		3.0	1 5	7
ANTI USERS TOTAL	9192	99	3153	9	627	21	729	35	1309
CONC. REGULAR	2	3	1		5		1	1	1
NOT	3.4	95 8	95	94	94.4	97	92	95.3	94.
NO RESPONSE	4.2	3.	4.4	5 3		2	7 2	4.6	3 0

TABLE 85 USE OF PHARMACEUTICALS WOMEN

	WOMEN			WOMEN			WOMEN		
	TOTAL	NC	CZ	26-35	36-45	46-	26-35	36-45	46-5
REGULAR INTAKE OF THE PHARMACEUTICAL	42	55	34	78	87	1	9 3	1	44
CONC. YES		3.		0	4		4.0	3 0	1
		4.	1	72 9	77 8	83	1	4.	
NO RESPONSE		5	2 1	12	14.	1	25	22	
RESTORATIVES	3		3 C	9 2		1	442	122	173
TOTAL	3 1				1		2	3.	4.7
CONC. REGULAR		4.		4.8	9.1	77	74.1	1.2	7
NOT REGULAR / NO RESPONSE	78.2	1	22	1		13	21 5	9	2 9
PRESCRIPTION-FREE ANALGESICS	42	150	3723	591	798	1	1		1675
CONC. REGULAR			2				1	7	
NOT REGULAR		94.2	5	94.	4.3	94.	94 8		94 1
		4.			1		5	4.5	.8
SLEEPING PILLS TOTAL	9	2	34 3	93	59	1893	44	1090	1837
CONC. REGULAR			1				1	2	
NOT REGULAR	97	47	97	94.	8.	9	5	4.	2
NO RESPONSE	1	5	2		1.3	1		3 0	1.8
TRANQUILIZERS TOTAL	60	2 1	3600	9 5	77	110	31	1143	1959
CONC. REGULAR					4		2		1
NOT REGULAR	93	3.	.8	89	93.1	95	99	90.	94 3
NO RESPONSE	.2	4.			4.3	1	1		5
ORAL CONTRACEPTIVES PRESENTLY									
TOTAL	34	252	3973	79	35	11 1	94	12 2	1714
CONC. YES		9.	2	5.2	9.1	1.4	1 9	1	8 7
NO	7	73.	71	94	9.	7	94 3		9.
NO RESPONSE	2	4.	7	11 2	23.		1 8	2	23.8
ORAL CONTRACEPTIVE PILLS NOW OR ...									
TOTAL	140	2347	3403	444	773	1123	9	113	1 16
CONC. YES	36.1	33.1	34	18	44.4	9	12	3.	3 8
NO	36.4	3 7	34 8	37 9	21.4		9.7	20.	37.8
DISCRETIONARY	27	23.2	30.4		3.2	1	34.	34.	27.4

T LE 84 SMOKING HABITS WOMEN

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46 58	WOMEN DZ 26-35	36-45	46-58
SMOKING DISCORDANCE TOTAL	6 44	5 5	4099	501	873	1219	1329	1 40	1777
CONC. NONSMOKERS	18 2	4.4	34 7	9	34 1	4 8	41 5	3 8	31 9
SMOKERS	37 6	9 7	26 9	28 8	43 1	40	2 9	4.1	1.3
PRESENT SMOKERS	24 0	29.9	2 8	14.8	29	27 4	12 3	22 9	29 1
FORMER SMOKERS	3	4.3	3 2	0	5 0	3 9	1 8	0	
DISC. S VRS NS	23 7	3	7 8	21 8	20	13 1	36 7	29	22 3
PRESENT S VRS NS	16 4	19	20 3	13 6	11 8	9	26 0	19	16 8
FORMER S VRS NS	7 2		7 8	9 2	8	2	8 6	10 3	9
PRESENT S VRS FORMER S	9 9	9 5	10 9	8 0	8 6	8 7	8 7	12 6	12 8
CIGARETTES TOTAL	6340	711	4129	605	877	1 26	3343	1299	[illegible]
CONC. CIGARETTES	36.8	39 3	37 8	28 6	4 4	40 1	21	4	2 2
NOT	38	4 1	35 5	49 8	36 5	49 7	2 5	31 1	3 3
DISC. CGT VRS NOT CGT	24 0	27 3	28 3	21 3	29.8	12 5	3 1	3	22 9
CGT VRS NS	23 1	14 8	27 2	21 2	20 3	12 4	33 8	29 3	21
CIGARETTES PRESENT									
CONC. CGT PRESENT	3 4	19 5	22 0	16 5	8 7	27 7	11 6	27 0	27 1
NOT CGT	53 9	9	46 8	41 7	50.	54 6	53 2	5 6	3 9
DISC CGT PRESENT VRS NOT CGT PRESENT	26 2	19.3	30 8	21 5	26.5	17 3	34 6	12 2	27 5
CGT PRESENT VRS NS	15	10.5	19 4	13 2	11 6	8 4	25 3	19 1	16.2
CIGARETTES QUANTITY									
1)CONC. 5 CGT	5	3.8	6 6	8	4 6	2 9	4 5	8 0	6 8
>10 CGT	1 1	6	1 3	8	4	6	1 0	1 9	1 1
2)DISC. 5 CGT	3 0	2.1	3 6	2 1	2 5	1 8	2 1	4 1	.2
>10 CGT	5	4	5	7	3	2	5	8	5
FILTER-CIGARETTES TOTAL	2521	1020	1501	174	370	476	253	509	739
CONC. WITH	8	9 7	83 9	47 2	85 1	97 3	95 7	0 4	95 9
WITHOUT	1 7	1 6	1 8	3 4	2 4	2	5 9	2	
WITH WITHOUT									
DISC. WITH VRS WITHOUT	9 7	8 9	10 2	23 6	10 5	2 3	22 9	13 4	3 7

1 PRESENT AND FORMER SMOKERS
2) PRESENT SMOKERS

TABLE 84 SMOKING HABITS WOMEN CONT D.

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46 58	WOMEN DZ 26-35	36-45	46-58
CIGARS/CIGAR-CIGARETTES	636	2544	3822	568	817	11	963	1202	1 54
CONC. CIGAR/CIGAR-CGT		4	2	2	4		2	2	2
NOT	9 3	94 9	93 9	94 0	93 8	6 0	94 6	94 2	1 7
DISC CIGAR/CIGAR CGT VRS NOT	3 2	4 4	5 7	3	5 6	3 2	5 1	5 5	2
CIGAR/CIGAR-CGT VRS NS	1 8	1 4	2 0	2 8	1 2	9	2 5	1 9	1 8
CIGAR/CIGAR-CGT PRESENT	7	3	1					2	1
CONC. PRESENT	99	5.0	95 3	95 6	95 0	96	94 3	95.3	9 8
NOT PRESENT	3	3 4	4 3	3 9	4	2	1 6	2	4.8
DISC. PRESENT VRS NOT PRESENT	1 4	1 1	1 6	2 1	1 0		1 7	1	1
PRESENT VRS NS									
PIPE TOTAL	6344	2531	3813	563	813	1152	959	11 9	1653
	3		2					2	
CONC. PIPE	97	8	97 6	99 6	98 9	7	99 3	98.2	96 1
NOT PIPE	1 8	1 3	2 1	4	1 1	1 9	7	1 6	3 5
DISC. PIPE VRS NOT PIPE	4	2	4		2	2	3	3	8
PIPE VRS NS									
PIPE PRESENT	1	1	1			3		1	2
CONC. PIPE PRESENT		9 2	98 3	9 8	99	9	9 4	8 7	7 5
NOT PIPE PRESENT	1	7	1	2		1 3	4	1 1	2 2
DISC. PIPE PRESENT VRS NOT PIPE PRESENT	3	1	9		1	1	3	3	7
PIPE PRESENT VRS NS									
SNUFF/CHEWING TOBACCO TOTAL	3 1	2532	3309	563	813	1153	9 8	11	1 51
		1							
CONC. SNUFF/CHEW TOB	9		9.4	99	9	99	9	100 8	99 5
NOT SNUFF/CHEW TOB	5		5		1		1		1 2
DISC SNUFF/CHEW TOB VRS NOT SNUFF/CHEW TOB		1		2		1			1
SNUFF/CHEW TOB. VRS NS									
SNUFF/CHEW TOB PRESENT		1							
CONC PRESENT	99 6	9	99.4	99	99 9		9	100 0	9.2
NOT PRESENT	3	3			1		1		8
DISC PRESENT VRS NOT PRESENT				2					
PRESENT VRS NS									
TYPE OF DISCORDANCE TOTAL	09	2	173	411	839	12	10 3	336	10 3
ONLY CGT VRS NS	1 8	1 8	23 1	17 7	17 7	11	28	29 3	10.2
ONLY CIGARS VRS NS	3	3		9				4	3
ONLY PIPE VRS NS									1
ONLY NC F VRS									

TABLE 87 PERSONALITY VARIABLES AND STRESS: MEN

	MEN			MEN			MEN		
	TOTAL			26–35	36–45	46–58	26–35	36–45	46–58
INSTABILITY, TOTAL	5	2279	3 70	911	715	10 2	812	1104	1752
CONC. SCORE 5–9	3 3	6.		5	3			4.3	1
0–4	72.3	74.	73.9	73	75.2	7	71 3	72 2	6
DISCORDANCE	22 2		2 3	20.	10.	1 2	7 3	2 5	2 7
DISCORDANCE 0–3/6–9	10.7	9	12	10 0	8.0	6 1	14 3	12	11
INTROVERSION-EXTRAVERSION, TOTAL	993	277	3640	508	715	1090	13	1104	17 3
CONC. SCORE 5–9	3 3	3	7 5	3 5	.6	84.4	37 8	3	
0–4	1	20.7		25	10.	19	21 2	20 3	1 3
DISCORDANCE	36 5	30.	40	33 1	33 7	2 8	1 1	2 3	39.1
DISCORDANCE 0–3/6–9	1 3	9	17 3	11	12		1 2	1	13 7
CONC. OF INSTABILITY AND INTROVERSION-EXTRAVERSION, TOTAL	59	237	16 1	51	71	1090	11	1102	1740
CONC. 5–9,0–4	2.0	2 8	1 7	2 7	1	3	1 7	1	1
5–9 5–9 2	1	1.0		1	3	7.6	6	5	1 3
0–4 5–9 3	11 3	13.2	1 8	16 2	12 3	12	12 5	12 7	7 2
0–4,0–4 4	36.1	40.3	3	12	3 .5	44	3 0	25.5	37 5
DISC. VS.	2.2	3.2	2 2	1	3.1	1	2 0	2	2.2
VS. 3	1		2		5.0	1		5	4.8
VS.	6.	4.7	7 7	9	5 3		7.0	7	8 2
2 VS. 3	2	2.1	3 3	2 3	2.3	2 2	3 1	2	3 7
2 VS.		7 1	0			.6	2	7 5	6.
3 VS.	2	20.	27 2	26.3	23.	1	2 9	29 7	2
DIFFICULTY IN FALLING ASLEEP TOTAL	5663	223	3625		29	19 1	61	1104	1720
CONC. YES	4.5	5.	3.6	2 6	4.5	.8	2 2	2 7	4.2
NO	72	75.9	70	6.	78.0	73 1	8	72	48
DISCORDANCE	23.1	18.3	2 8	18.9	2 5	2 1	23 7	2	1
STRESS-FILLED DAILY EXISTENCE, TOTAL	58 3	2222	35 3	494	7 3	1 24	798	207	72
CONC. YES	5 9	8	5 3	8 9	7 3	3 9	3 9	6.	.2
NO	73 5	72.1	48	66.	8.	79	64.	3.	7
DISCORDANCE	22.6	20.1	29 8	24 9	24.8	15.2	29 7	38 9	21.

TABLE 87 PERSONALITY VARIABLES AND STRESS: WOMEN

	WOMEN			WOMEN			WOMEN		
	TOTAL			26	36–4	46–5	26–35	6–45	4– 5
INSTABILITY, TOTAL	.66	272		60		122	0	294	7
CONC. SCORE 5–9	1			1	7		1	1	2
0–4		40.		1	44		7	57 9	3
DISCORDANCE	2	4.3	3	24	2 9	3	3	1	35 6
DISCORDANCE 0–3/6–9	1		1		1				5
INTROVERSION-EXTRAVERSION, TOTAL	.47	27	136	68	1	1	04	294	2 86
CONC. SCORE 5–9	31	34 1	28	30.	3.	40.	1		3 3
0–4	31		29.3	38 1	.6	32	2	1	27 5
DISCORDANCE		2 1			2		48	44.8	3 .2
DISCORDANCE 0–3/6–9		5			11.0		23	9	5
CONC. OF INSTABILITY AND INTROVERSION-EXTRAVERSION, TOTAL		171	4.	40		1275	134	129	1740
CONC. 5–9,0–4		3.	2.2		1.				
5–9,5–9	1	4.	.2	20	1 .2	1 .6	1 2	3.	1
0–4 5–9	21.6	4.		22.	2	2	1 7	1	
0–4,0–4				4.1				2	3.
DISC. VS.	0.	4.	1	10.			1 .6	10.	1
VS.			10 2	6.			11	10.	2
VS.				1.		1	3	4.	3.
2 VS.		4.							
VS.		14.		1	3	14.	2	2	10.
3 VS.									
DIFFICULTY IN FALLING ASLEEP TOTAL	77	3	1	3 8	8.3	12	10	1294	771
CONC. YES		3		4.9		1	1		
NO		9.	62	64.	72.	44			5 7
DISCORDANCE		2 2	34.	26.	3	21	1	26.	12 7
STRESS-FILLED DAILY EXISTENCE, TOTAL	24	242	4036	849	54	18	132	12	1
CONC. YES		5.		4.3	3.		1 9	2	
NO	77 5	4.8	7	71	7 .6	88 1	7	79	74.6
DISCORDANCE		4.	3 1	22	3 1	13	23	18.2	1 .2

TABLE 86 DRINKING HABITS MEN

	MEN TOTAL	MEN MZ	MEN DZ	MZ 26-35	MZ 36-45	MZ 46-58	DZ 26-35	DZ 36-45	DZ 45-58
ALCOHOL CONSUMPTION EXPRESSED AS GRAMS OF 100 % ALCOHOL/MONTH TOTAL	3744	506	2258	309	450	4	4 5	454	1139
CONC 000 -0	24 0	26 2	22 5	20 4	10 2	34 8	15 1	4.7	27 9
001 250 1	13 6	15 5	12 4	11 0	18 9	15 3	11 4	13 6	1 1
251 500 2	8 4	7	7 6	4 8	11 3	9 4	5 4	6 1	4 2
501-750 3	2 1	1 9	2 2	2 3	2 2	1 5	1 3	2 8	2 3
751 1000-4	5	7	4	6	4	5			4
1001 5	9	1 3	7	2 4	1 3	7	1 3	9	
DISC. 0 YRS 1	10 1	8 8	10 9	13 6	9 6	6 4	1 6	12 1	8 0
0 YRS 2	3 7	2 7	4 4	4 2	2 9	2 3	5 4	1	4.1
0 YRS 3	1 4	7	1 8	1 9	9	1	2 2	2 4	1 1
0 YRS 4	6	3	8	6		1	6	1 2	5
0 YRS 5	5	2	7		4	1	1 3	3	
1 YRS 2	13 4	12 2	14 3	11 7	17 3	4	14 3	21 3	12.
1 YRS 3	4 2	3.9	4 4	5	5 3	2 9	6 0	4 0	2 9
1 YRS 4	1 8	1 9	2 0	2 3	1 6	1 1	3 0	2 3	1
1 YRS 5	1 9	7	5 9	1 5	7	9	3	2 4	1 0
2 YRS 3	6 1	7 1	5 4	7 8	9 1	8 5	8 6	4 0	9
2 YRS 4	2 0	1 5	2 3	2 3	1 1	1 3	2 4	3 2	1 8
2 YRS 5	1 8	1 9	1 9	2 3	1 3	1 5	1 9	2 4	1 8
3 YRS 4	1 3	9	1 5	1 0	9	9	1 5	1 1	1.8
3 YRS 5	1 4	1 7	1.2	1 9	2 4	1 1	1 3	1 2	1 1
4 YRS 5	9	1 1	8	1 0	1 6	8	9	8	8
ALCOHOL DIFFERENCE EXPRESSED AS GRAMS OF 100 % ALCOHOL/MONTH TOTAL	3763	1506	2257	309	450	746	465	453	1139
000- 500	88 6	1 5	86 7	88 0	83 1	94 4	83 0	83 2	0.2
501 1000	8.7	6 7	10 1	9 6	9 1	4 2	12 0	12 9	7 3
1001 1500	1 8	1 3	2 1	1 6	1 6		3 2	2 5	1 5
150 2000	5	2	7	3		3	1 3		
2001-	4	4	4	6	4	3	4	8	2
"MORE THAN HALF A BOTTLE" ON THE SAME OCCASION TOTAL	5452	2173	3479	490	680	94	763	1094	16 5
CONC. YES	9 0	9 8	8 5	5 7	7 3	13 4	2 2	9	13 8
NO	69 5	71 3	68 4	78 2	73 8	66 1	80 9	72 8	59 8
DISCORDANCE	21 5	18 9	23 1	16 1	18 9	20 3	16 9	22 3	26

TABLE 86 DRINKING HABITS WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	MZ 24-35	MZ 36-45	MZ 46-58	DZ 26-35	DZ 36-45	DZ 46-58
ALCOHOL CONSUMPTION EXPRESSED AS GRAMS OF 100 % ALCOHOL/MONTH TOTAL	177	1 7	29 1	358	512	3	6 5	7	11C
CONC 000 -0	1 (	53 2	0 5	47 2	31 6		7 1	32	7 8
001 250 1	20	23 2	19	12 3	26 0	26	10 3	1	23 3
251 500 2	2 9	5	2 1	3 9	3 3	4	1	7	7
501 750 3	3		2	6			8	1	
751 000-4	1	1	1				2	3	
00 5		1							1
DISC 0 YRS	18 5	1 0	20 8	19 6	18 8	10	25	3 0	1 7
0 YRS 2	2 8	1 6	3.4	2 5	2 7		5 1	3	2 0
0 YRS 3	5	3	4	8	2			7	
0 YRS 4	1	1	2		2		3		2
0 YRS 5	2	1	2	3		1	3		
1 YRS. 2	8	7 9	8 7	8 9	10.7	5		10 8	9 C
1 YRS 3	1	1 1	1 6	1 7		1	2 0	1	1 3
1 YRS	2	1					1		
1 YRS 5	2	2	2		7		2		8
2 YRS 3	1 2	1 4	1 1	1	3	1	8	1	1 C
2 YRS			4				3		2
2 YRS 5	1	2	1	1		1			1
3 YRS	1	1	1	1		1		1	1
3 YRS 5	1	2		3	2				1
YRS 5									
LCOHOL DIFFERENCE EXPRESSED AS GRAMS OF 00 % LCOHOL/MONTH TOT L	1 7	1474	2523	358	512	5			1109
00- 500	8	9 0	77	9 5	98	0	94	0	
501 1000	1 8	1	2 0	2 2	1 0	1		2	1
1001 500		3					9	1	9
50 000	1	1		3		1			1
2001									
"MORE THAN HALF A BOTTLE" ON THE SAME OCCASION, TOT	65 0	2	3 3	5 8	83	118	5	1 3	177
CONC. YES	1 3	1 5	1 1	7		2	2	3	7 1
NO	3 1	1	92	94.1	45 3	3		94	88 3
D SCORDANCE	9	4 4	6 5	3 2	4 3	5 0	3 2	1	3

TABLE [illegible] FOOD HABITS, MEN CONT'D. Z

	MEN Z [illegible] Z			MEN Z			MEN Z		
	TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FRUIT TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1 [illegible]
1) CONC. YES	70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7
NO	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	17.0	[illegible]	[illegible]	[illegible]
2) PRONOUNCED DISC.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
MILK, [illegible] MILK, YOGHURT OR CHEESE TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CONC. YES	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
NO	[illegible]	7		[illegible]		[illegible]		[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11	[illegible]	7
PRONOUNCED DISC.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] MEALS PER DAY TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CONC. [illegible] MEALS	[illegible]	19	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	17.4	[illegible]	[illegible]	[illegible]
DISCORDANCE 1/2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] PER DAY TOTAL	[illegible]	2227	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1700
CONC. [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]	[illegible]
DISCORDANCE 4/5	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
COFFEE PER DAY TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CONC. [illegible] CUPS	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	12	[illegible]	21	1
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	[illegible]
DISCORDANCE 4/5	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]	[illegible]
POTATOES PER DAY, TOTAL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CONC. [illegible] POTATOES	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DISCORDANCE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DISCORDANCE 4/5	[illegible]		[illegible]	[illegible]	[illegible]		[illegible]		[illegible]

1) AT LEAST SEVERAL TIMES PER WEEK.
2) THE ONE TWIN IN THE PAIR STATED AT LEAST SEVERAL TIMES/WEEK, THE OTHER STATED [illegible] [illegible]

TABLE E8 FOOD HABITS MEN

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 26-35	MEN MZ 36-45	MEN MZ 46-58	MEN DZ 26-35	MEN DZ 36-45	MEN DZ 46-58
GRILLED OR DEEP-FRIED FOOD TOTAL	51 0	2019	3171	426	629	964	61	9 3	1 96
1) CONC. YES	2 3	3.0	1 8	7	1 4	5 0	5		2 8
NO	84 5	85 3	83 9	90.8	85 3	82 7	90 6	84 5	80 7
DISCORDANCE	13 3	11 7	14 3	8 5	13 0	12 3	9 0	1 1	16
2) PRONOUNCED DISC.	8 7	7 2	9 6	7 0	8 6	6 3	7 3	10 3	10 0
PAN-FRIED OR ROASTED FOOD, TOTAL	5685	2187	3498	494	696	999	776	1058	1664
CONC. YES	55 3	57 3	54 1	55 9	57 1	58 3	55 2	55 6	2
NO	11 0	12 2	10 3	10 5	10.1	14 5	5 2	5 9	14 1
DISCORDANCE	33 6	30 5	35 6	33 6	32 9	27 2	34 6	38 7	33.3
PRONOUNCED DISC	4.6	4.5	4 7	4 5	5.6	3 7	4 0	7	5 0
PURE MEAT TOT L	5716	2200	3516	492	694	101	760	1043	1691
CONC. YES	36 2	39 7	34	39 6	39 2	37 9	39 9	34 4	32 4
NO	25 8	26 5	25 3	18 9	22 8	32 9	18 3	21 6	30 8
DISCORDANCE	39 1	34 8	40 1	41	38 0	2 3	41 9	44 0	36 8
PRONOUNCED DISC.	7 8	7 0	8	7 7	7 2	6	6 8	9 3	8 5
SAUSAGE OR HOTDOGS TOTAL	5771	2215	3556	491	698	1026	765	1080	1711
CONC. YES	18 5	20.5	17 4	14 7	18 9	26 3	13 6	11	2 8
NO	45 5	46 1	45 2	47 0	45 9	43 2	49 9	9 2	40 5
DISCORDANCE	35 9	33.4	37 5	38 3	34 2	30	36 5	3	36 7
PRONOUNCED DISC.	8 3	7 5	8 8	10 2	7 6	6 2	8 8	10.7	7 5
LIVER KIDNEY, BLOOD OR OTHER ORGAN FOOD, TOTAL	5672	2193	3479	486	691	1016	732	1058	1669
CONC. YES	9	7	1 0	8	6	8	5		1
NO	91 5	91 9	91 2	88 9	93 1	2 6	90 0	9 9	90
DISCORDANCE	7 6	7 3	7 8	10 3	6	6 6	9 4	6 7	7 8
PRONOUNCED DISC.	4 7	4.1	5 0	5 8	3 8	3 5	6 5	4 8	4 5
FISH TOTAL	5824	2235	3589	508	704	1023	794	1087	1708
CONC. YES	9 8	11 1	9 0	11 6	8 4	12 5	7 3	7 0	11 0
NO	59 3	61 0	59 3	55 7	62 4	62 7	53 9	58 9	60 0
DISCORDANCE	30 9	28 0	32 7	32 7	23 3	24 7	33 8	34 2	29 8
PRONOUNCED DISC.	6 6	5.3	7 4	4 7	3 7	5 4	8 2	7 8	6 7

1) T LEAST SEVERAL TIMES PER WEEK
2) THE ONE T IN IN PAIR ST TED AT LEAST SEVER L TIMES/WEEK; THE OTHER STATED ONE OR TWO TIMES/MONTH T MOST

TABLE E8 FOOD HABITS MEN CONT D I

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 26-35	MEN MZ 36-45	MEN MZ 46 59	MEN DZ 2 35	MEN DZ 36-45	MEN DZ 4 59
SHELLED SEAFOOD TOTAL	5440	2109	3331	4 9	659	991	658	1030	1633
1) CONC. YES	1	3	1		3	4	1		
NO	96 0	97 2	9 8	95 8	97 0	97	97 1	96.9	9 6
DISCORDANCE	2 9	2 6	3 2	3 1	2 7	2 2	2 8	3 1	3
2) PRONOUNCED DISC.	2 4	1 9	2 7	2 4	1 0	1 7	2 6	2 8	7
RICE AND RICE DISHES TOTAL	5590	21 9	3 31	72	678	1009	716	1C 5	1672
CONC. YES	1 9	2 2	1 7	1 7	9	3 3	4	9	2
NO	86 9	85 6	87 7	86 9	84 5	85 7	90 9	8	8
DISCORDANCE	11 3	12 2	10 6	11 4	14 6	11 0	8 5	12	10.5
PRONOUNCED DISC.	6 3	4.3	6 3	5 9	7 2	5	5	7 2	0
FLOUR-BASED FOOD (PORRIDGE DRY CERE L PANCAKES ETC) TOT L	57 1	2219	3572	9	695	1033	772	1087	1713
CONC. YES	17 6	20 0	16 2	17	11 1	25 8	14	11	26.1
NO	9	4 9	9 0	49 0	54 2		8 3	52	7 2
DISCORDANCE	33 0	30 2	34 7	33 6	32 7	2 9	3 5	36 2	32 7
PRONOUNCED DISC.	13	12 2	14 8	15.0	13 8	8	17 2	1 3	12 8
EGGS AND EGG DISHES TOTAL	5810	2235	3595	499	732	1036	787	1093	1715
CONC. YES	30 1	0 6	29 8	31 5	29 1	31 1	28 3	29	33
NO	30 7	3.8	28 8	2	33 3	38 5	25 5	2	31 5
DISCORDANCE	39 2	35.7	41 3	3 9	37 6	35	1	3	37 5
PRONOUNCED DISC.	11 1	5	12 0	13 0	7 3	9	1	13.3	10 3
VEGETABLES AND ROOT VEGETABLES TOTAL	5830	2249	3585	504	706	1035	793	1087	1713
CONC. YES	59 1	60.2	58	57 9	61 5	0 5	5 5	59	57 5
NO	10 2	12 3	8 9	10.9	9 8	1 7	8 2	7 2	10 3
DISCORDANCE	30.7	27 5	32 7	31 2	28 8	2 9	37 3	33 2	30
PRONOUNCED DISC.	8 6	6.9	9 7	8 1	7 2	6 2	10 7	8 7	9 9

1) AT LEAST EVERAL T MES PER WEEK
2) THE ONE TW N IN THE P ST TED LE ST EVERAL ME /WEE ; THE OTHER ST ED ONE OR TW ME /MONTH MOST

TABLE 88. FOOD HABITS, MEN CONT'D. 2.

	MEN			MEN			MEN		
	Z 0 Z			Z			0 Z		
	TOTAL	MZ	DZ	26 35	36-45	46-58	26-35	36-	46-58
FRUIT TOTAL	8018	223	3404	5	733	1 36	793	1094	1727
CONC, YES	0	1 3	9	63	8.	7 5	42	7.8	7 6
NO	5 7	8	5.8	7 9	7 0	2		4.	4.9
INDIFFERENCE	23.	21	25 1	2 7	24.9	17	81	28.2	28 5
2) PRONOUNCED DISC.	7 8	4.8	8 5	11 7	4.5	4 8	12 0	11 2	5 2
MILK, SOUR MILK, YOGURT OR CHEESE, TOTAL	5901	2254	36 7	507	718	1037	800	1185	1739
CONC, YES	91 3	92 3	90 6	89 /	51 5	94 1	7 8	98 0	2
NO	5	7		1 2					3
INDIFFERENCE	8 2	7 0		1	8.0	5 3	11		7 3
PRONOUNCED DISC.	2 7	2 2	3 0	3 4	2.4	1 5	3 3	3	2 7
MAIN MEALS PER DAY TOTAL	5 22	2232	3590	540	702	1029	788	1092	1 1
CONC, 0-1 MEAL(S)	1 3	19.7	1 1	20.0	25.3	15 5	21 1	25 5	1 .0
3-	54 8	56.2	52.4	49 8	44	1	44	40.0	3 3
INDIFFERENCE	26 7	24.1	2 4	30 2	29.6	17	32 1	34	22 7
INDIFFERENCE 1/2	25 1	22 8	25 5	26.	28 1	15 5	2	31 9	2
SANDWICHES PER DAY TOTAL	5802	2227	3575	87	732	1 27	7 7	1048	1760
CONC, 9- SANDWICH(ES)	39 2	1 2	37 9	32 0	4.5	50.2	31	30	49 3
0-4	29.1	28.	23.0	34.	30.6	24 1	26 2	26 2	1 5
INDIFFERENCE	38 8	30.4	39 1	33	34 9	25 7	1 9	2	3 .2
INDIFFERENCE 4/5	7 2	7 3	7 1	8	8 0	4 5	4	7	4.8
COFFEE PER DAY TOTAL	5738	2209	3 29	492	697	103	784	1091	164
CONC, 5- CUP(S)	1	20.3	18 8	32 3	23.8	2	29	21 8	18
0-4	1.0	59 2	48.	37 0	1	70.4	28	34	65 3
INDIFFERENCE	30 1	24.5	33 6	30.7	31 0	1 1	2	1 9	2 3
INDIFFERENCE 4/5	5 1	5 1	5 1	4.3	7.8	3 2	3	5	4.1
POTATOES PER DAY TOTAL	5778	2221	3557	498	97	1 25	84	1092	1 1
CONC, 5- POTATO(ES)	39	30 5	3 7	29	33 3	40	20.	8.7	1
0-4	30.7	32.5	29		34.	2 .4	37	29	25 8
INDIFFERENCE	3.7	29 0	34	32 7	31 9	25 4	1.2	39 7	32.6
INDIFFERENCE 4/5	8.1		8 2	10.0	8.2		9	8.	7 9

*) AT LEAST SEVERAL TIMES PER WEEK.
2) THE ONE [illegible] IN THE PAIR STATED AT LEAST SEVERAL TIMES/WEEK THE OTHER [illegible] OF TWO [illegible]/MONTH AT MOST

TABLE D8. FOOD HABITS WOMEN

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
GRILLED OR DEEP-FRIED FOOD TOTAL	970	2 9	3941	4 7	790	1140	794	111	1629
1) CONC. YES	8	1 1	5	4	6	1 8	1	2	9
NO	92 7	93.2	92	3 8	93 2	93 0	94 6	91 2	92 1
DISCORDANCE	6 5	5.6	7 1	5 8	6 2	5	5 3	2 4	7 0
2) PRONOUNCED DISC	4 5	3.6	5 1	4 0	4 2	3 0	3 6	4 9	6
AIR-FRIED OR ROASTED FOOD TOTAL	65	2403	3936	548	838	11 5	938	1226	1724
CONC YES	6 3	59 0	54 5	64 3	61 2	55 1	63 4	4 1	7 7
NO	13 0	14 0	12 3	8 3	9 8	19	6 7	8	18 1
DISCORDANCE	30 7	27 0	33 2	27 5	24 0	25 1	32 9	32	34.0
PRONOUNCED DISC.	4 2	3.6	4 6	3 5	4 2	3 1	4 7	3 8	5 1
PURE MEAT TOTAL	6 10	2428	3982	5 3	840	1201	99	12	1731
CONC YES	35 0	36.6	35 5	42 2	43 4	31 3	45 8	39 7	26
NO	28 2	31 4	26 1	19	23 7	4 5	15 3	19 1	37 3
DISCORDANCE	35 9	32 0	38 4	38 6	36 0	26 1	38 8	41 2	36 1
PRONOUNCED DISC	7 1	6.0	7 8	5 3	6.3	6 0	7	7 7	1
SAUSAGE OR HOTDOGS TOTAL	6627	2441	3986	572	856	1217	995	12 7	1732
CONC YES	12 9	7	11 8	10 1	11 8	18	11 3	8 7	1 .2
NO	56.3	57 7	55 4	55 9	58 3	59 1	51 9	4 3	56 1
DISCORDANCE	30 7	2 6	32 8	33 9	29 9	22	36 6	35 0	29 1
PRONOUNCED DISC.	7 9	6.5	8 9	4	6 7	5 0	9 9	10 7	7 8
LIVER KIDNEY BLOOD OR OTHER ORGAN FOOD TOTAL	6577	627	3950	76	8	1232	955	1217	1776
CONC. YES	9	1 4	5	1 6	1 5	1 3	4	6	1
NO	80 1	90.9	69 6	88 6	85 5	91	86 0	88 7	82 7
DISCORDANCE	9 0	7 7	9 9	9 9	9 9	5 0	13 4	10 7	7 4
PRONOUNCED DISC	5 2	4 4	3 8	4 7	6 0	3 1	7 4	6 3	5
FISH, TOTAL	6734	2648	4034	588	864	121	1030	1259	17 5
CONC YES	12 9	24 3	22 9	24 6	12 4	28 4	12 6	12 1	12 3
NO	53 5	56 6	51 4	46 9	56 4	61 5	44 5	48 2	57 7
DISCORDANCE	33 6	29 0	36 6	35	31 3	22 9	42 8	39 7	30.3
PRONOUNCED DISC.	6 3	4 8	7 2	8	3 6	4 8	7 9	7 1	6.9

1) AT LEAST SEVERAL TIMES PER WEEK
2) THE ONE T N I THE PAIR STATED AT LEAST SEVERAL TIMES/WEEK; THE OTHER STATED ONE OR TWO TIMES/MONTH AT MOST

TABLE D8. FOOD HABITS WOMEN CONT'D

	WOMEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
(E FOOD TOTAL	63	2964	3819	595	826	1201	908	1195	1715
1) CONC. YES	8	6	1		7		1	2	1
NO	96 7	9	96 8	9 2	9 8	95 5	7 0	9	97 9
DISCORDANCE	3 0	2 8	3 0	2 8	3 5	2	2 9	1	2 4
2) PRONOUNCED DISC	3	2 0	2 6	1 9	2 5	1	2 9	1 6	1 7
CE AND CE DISHES TOTAL	6502	2599	3903	365	8 3	1203	958	121	1729
CONC YE	1 8	2 6	1 4	2 5	1 9	3 1	9	1 2	1 7
NO	85 3	85 3	85 2	6 0	81 2	86	86 0	3	89 9
DISCORDANCE	22	12 1	13	12 7	1 1	9	13 0	1	12 5
PRONOUNCED DISC.	7 3	4.7	7 8	6.9	8 3	5	9 0	8	6 6
BAKED FOOD, DRY CEREALS (PANCAKE ETC.) TOTAL	6 7	2462	401	588	861	1211	1004	1258	1750
CONC YE	1 0		11 8	1 3	13 5	1 7	15 1	12 9	12 9
NO	5 2	0	5	51 7	53.2	62	46 2	40 6	47 2
DISCORDANCE	31 7	2 1	1	33 0	33 3	22 0	38		8
PRONOUNCED	13 4	11 8	14	13.8	13 0	13 0	15 7	1 5	13 5
EGGS AND EGG DISHES TOTAL	66 2	61	031	78	86	121	1316	125	1 5
CONC. YES	31 6	3 0	3 0	33 0	3 2	32 1	35 5	3 7	10 8
NO	26 0	2 9	26 7	2	23 2	37 2	1 0	22 0	3 3
DISCORDANCE	39	3 1	3 3	42 6	2 6	30 4	2 5	1 3	35 9
PRONOUNCED DISC.	10.	7	11 7	12 5	9 9	8 3	10 8	2	11
VEGETABLES AND ROOT VEGETABLES TOTAL	67	691	079	5 9	8 7	122	1823	1240	1771
CONC YES	75 2	76 0	7 7	73 1	75 7	77	72 7	77 0	7 1
NO	5	4 7	3 8	6.0	6	4	2 7	2 5	.2
DISCORDANCE	20 3	19 3	21 4	20 9	19	1 0	2 5	20 5	18
PRONOUNCED DISC.		3 9	3 1	3 7	3 8	3 9		8	5

1) AT LEAST SEVERAL TIMES PER WEEK
2) THE ONE IN THE PAIR STATED AT LEAST SEVERAL TIMES/WEEK; THE OTHER STATED ONE OR TWO TIMES/MONTH AT MOST

TABLE 38. FOOD HAB TS WOMEN, CONT'D. 2.

		WOMEN			WOMEN			WOMEN	
		Z DZ			Z			DZ	
	TOTAL	NZ	DZ	2 35	36-45	46-58	26 35	36-45	46- 9
FRUIT TOTAL		449	43	40	71	2	97	127	177
D EXC. YES	6		87	89.6	87.4	8	95 2	1	
NO	1.4	2.2	1 1	2 3	3	3	1 1		1 5
INDIFFERENCE	10.	9.3	11 2	5	10.8		13	10.	1 3
B PRODUCES DISC.	3 2	4	3 5	6	3 1	1	8		2 7
MILK, SOUR MILK, YOGURT OR CHEESE TOTAL	818	788	110	499	87	12 7	1347	12	1 77
EXC. YES	3 1	49	9 9	44 5	3 3	95	94 2	5.	44
NO	2	1		3		2		1	
INDIFFERENCE	4.4	3		4.2		1	5	1	5.0
PRODUCES DISC.	1 4	3	3 1	1	1 3	1	3	1	2.3
MAIN MEALS PER DAY TOTAL	784	50	4084	601	465	1 2	1034	128	1 7
EXC. 0-1 MEALS	2 8	2	2 8	28.1	33 2	34	23 1	2 1	30 8
3-	3 8	3.7	1 2	40.	3	44	40	3.	2 3
INDIFFERENCE	30.	4.8	3	32 1	31 9	21	3 3	37 1	77
INDIFFERENCE 1/2	23.1	25 8	30.2	29	2	23.7	33 7	1	25 5
SANDWICHES PER DAY TOTAL	9	2441	40	49	46	12	1034	127	1 1
EXC. 5- SANDWICHES	1	3		4.		13	3	1	
0-1	71 7	2 7	71 8	75 3	73	7	7 8	4.	64
INDIFFERENCE	21 3	0	23	1 7	10 3	1	2 1	21	5 5
INDIFFERENCE 4/5	2	5.	5 2	8	3 7			5	5 1
COFFEE PER DAY, TOTAL	172	24 2	077	46	464	1204	1 11	1271	1 2
EXC 5- CUPS		7	16 3	2	23 8		34 1		8
0-4	93 3	45.	9 9	3	7	7	3	44	8
INDIFFERENCE	2.4	2 8	1	30.2	27 3	1	3		2
INDIFFERENCE 4/5		8			3 3			4.1	2
POTATOES PER DAY, TOTAL	7		48	49	864	121	3 3	127	179
EXC. 5- POTATOES	3		2	2	3 3		1		0
0-4	84		8 1		8.8	9 .3	1	94.	
INDIFFERENCE	1 9	4.8	1 3	8	9.8		11 8	8 3	
INDIFFERENCE 4/5	2 5	1.5			2 1	3	1 8	1	3

1) AT LEAST SEVERAL TIMES PER WEEK
2) THE ONE TWIN IN THE PA STATED AT LEAST SEVERAL TIMES/WEEK THE OTHER STATED ONE OR TWO TIMES/MONTH AT MOST

TABLE 89 — ANNOYANCE REACTIONS AT PLACE OF WORK, MEN

	MEN TOTAL	MEN M Z	MEN D Z	MEN M Z 26-35	MEN M Z 36-45	MEN M Z 46-58	MEN D Z 26-35	MEN D Z 36-45	MEN D Z 46-58
NOISE TOTAL	5 44	2047	3247	443	601	02	731	1032	1 1
CONC ANNOYED	18 7	19 5	18 4	17 7	19 4	20 6	17 1	19	18 1
VERY ANNOYED	3 3	3 3	3 3	1 5	2 9	4 5	3 8	2	2.8
1) DISC. 1	14.7	14.9	17 8	19 1	14 2	13	19 7	11 5	17.4
2) 2	10 2	8 0	11 5	8 6	7 6	7 4	12 6	12 8	10.2
VIBRATIONS TOTAL	5118	1988	3130	442	661	884	704	9 1	1445
CONC. ANNOYED	3 8	4 6	3 4	4 8	3 9	5 0	2 7	3 5	3.4
VERY ANNOYED	4	5	3	2	3	7	3	6	1
DISC 1	13 8	12 0	15 0	12 7	11 5	12 1	16 1	14 7	14 7
2	4 7	4 0	5 1	3 2	3 8	4 5	6 1	5.1	4.6
DUST SOOT SMOKE OR GASES TOTAL	5229	2024	3 05	449	673	01	721	1019	1445
CONC. ANNOYED	12 2	12.9	11 8	10 2	13 5	13 9	8 9	14 1	11 6
VERY ANNOYED	1 9	2 1	1 8	9	2 4	2 4	1 8	2 7	1 6
DISC. 1	19 6	17 1	20 9	18 5	18 9	19 2	24 7	20.3	19 3
2	9 9	7 8	11 2	8 0	9 4	6 4	11 1	11 1	10 8
ODOR TOTAL	5082	1976	3106	440	651	884	701	971	1 34
CONC. ANNOYED	4 5	4 4	4 5	3 9	4 6	5 0	3	3 8	3 3
VERY ANNOYED	6	6	6	5	5	9	6	4	7
DISC. 1	14 8	12 1	16 5	9 8	13 7	12 2	17 7	17 7	15.1
2	5 0	4 0	5.6	3.9	4 9	3 5	6 1	7 6	8
POOR/GLARING LIGHTING TOTAL	5143	1990	3153	443	664	882	709	999	1 31
CONC. ANNOYED	2 8	3 2	2 6	2 0	2 9	4 0	2 1	2 3	2 6
VERY ANNOYED	3	5	3	8	5	5	1	3	5
D SC. 1	15 6	13 6	16 8	15 8	13 9	12 4	15 8	19 2	13
2	4.2	3 8	4 3	3 2	5 4	2 8	3 3	6 6	3.4

1) THE ONE TWIN IN THE PAIR STATED THAT HE WAS ANNOYED; THE OTHER STATED THAT HE DID NOT NOTICE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT HE WAS VERY ANNOYED; THE OTHER STATED THAT HE D D NOT NOTICE ANYTHING ANNOYI F OR THAT HE NOTICED BUT WAS NOT ANNOYED

TABLE 89 — ANNOYANCE REACTIONS AT PLACE OF WORK MEN CONT D.

	MEN TOTAL	MEN M Z	MEN D Z	MEN Z 26-35	MEN Z 36-45	MEN Z 46-58	MEN D Z 26-35	MEN D Z 36-45	MEN D Z 46-58
UM O TY TOTAL	5099	1981	3118	439	663	878	701	981	1 5
CONC. ANNOYED	1 6	2 0	1 3	1	1 5	1 6		1 6	1 3
VERY ANNOYED	2	3	1		2	6	1	2	1
) DISC 1	10 2	9 5	11 3	3	10 7	6	12 3	13 1	9 5
2) 2	3 0	2 3	3 4	1 0	2 6	1 8	3 7		2 6
NTENSE TEMPERATURE CHANGES TOTAL	5198	1993	3165	4 1	665	81	719	995	1 31
CONC. ANNOYED	5 6	1	4 4	5 7	7 2	7 7	3 2	5 2	5 5
VERY A NOYED	7	1.2	4	5	1 2	1 4	3	7	8
D SC 1	27 2	25 0	28 4	27 5	18 5	12 5	18 6	18.7	18 1
2	7 1	5.9	7 8	5 9	6 9	5 2	8 9	8 7	6 5
TOO HOT TOTAL	5115	1990	3125	443	662	854	712	974	1435
CONC. ANNOYED	3 9	4.2	3 6	2 3	4 4	5 0	2 7	2	3 4
VERY ANNOYED	7	1 0	5	2	8	1 6	6		4
D SC 1	18 2	16 6	19 3	17 6	18 0	15 0	21 3	20 8	17 3
2									
TOO COLD TOTAL	539	1979	3105	37	661	891	703	978	1 2
CONC. ANNOYED	3 5	4.2	3 0	3 0	4 5	5	2 7	3 8	2 7
VERY NNOYED	5	8		5	5	1 2		5	2
D S.. 1	16.8	14.8	18 0	14.5	16 3	12 8	18 6	18 8	17 1
2	5 5	5	2	1	5 1	2	7 6	6.2	5.5
UNSUITABLE WORK POSTURES TOTAL	5341	1972	3109	36	836	479	697	980	1 32
CONC. ANNOYED	7	7 8	7 1	6	5 3	10 2	2	7 1	8 8
VERY ANNOYED	7	1 1	5	7	1 1	1 3	1	6	6
D ... 1	19 1	16.5	20 8	16 3	19 1	1	25 2	20 7	20.6
2	7 0	5.7	7 8	9 8	6	5 3	8 3	8 6	7 1

) THE ONE T THE IR ST TED THA HE S ANNOYED; THE OTHE TED THA HE D D NOT NOT CE ANY ANNOYANCE

2) THE ONE TW THE AIR ST TED THA HE S VERY ANNOYED; HE OTHE ST TED THA HE D NOT NOT CE ANYTH NG ANNOY NG OR THA HE NOT CED BUT S NOT NNOYED

TABLE 98. ANNOYANCE REACTIONS AT PLACE OF WORK, WOMEN

	WOMEN % TOTAL	% WZ	% DZ	WOMEN % 26-35	36-45	46-58	WOMEN % 26-35	36-45	46-58
NOISE, TOTAL	1	1900	2596	346	536	44	544	721	1290
CONC. ANNOYED	3.9	4		0	7	1 4	2 2	3 3	4 3
VERY ANNOYED		1 1	7	3		1	2	7	
DISC. 1	14 0	12 8	14	11 0	1 2	11	1	1 1	13 5
2	5 1		5 6	4.1		4		1	5 0
VIBRATIONS, TOTAL	4851	1 59	2 52	357	448	1	52	781	1259
CONC. ANNOYED		1.2	7	4	1	1 5		3	
VERY ANNOYED	1	2	1	3	2	1		1	1
DISC.	5.	5 3	8	7	8 9	3	5 7	8 3	1
2	1 1	1 0	1 2	9	8		1 5		1
DUST, SOOT, SMOKE OR GASES, TOTAL	242	1781	2511	345	92		33	707	1275
CONC. ANNOYED	2 1	3.9	2.6	1 7	2		2 1	1 7	3
VERY ANNOYED			3			9		8	
DISC.	1 1	13.3	1 7	15	11 2	12	1	1	1 8
2		4.	7	9	3	3	5	9 3	3
ODOR, TOTAL	244	175	2 55	338	35	3	513	44	1265
CONC. ANNOYED	2 1	3.1	2 5		2 1		2 3	1	3.2
VERY ANNOYED	3	5		3	2	9		1	3
DISC.	11 8	10.	12	2	10.3	1 2	12	13 1	12 5
2	2.	2.5	3.2	3.0	2 1	2	3		2
POOR/GLARING LIGHTING, TOTAL	247	1773	1	341	5		93	704	1276
CONC. ANNOYED	2 8	3	2 3	2 9	1		1	2.	2
VERY ANNOYED		3	2		2			1	2
DISC.	12	1	13.7	11 7	11 9	1	13	13 1	1 1
2	2 5	8 2	2	1	3 8	1	2 1	2.8	2 8

THE ONE TWIN IN THE PAIR STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

TABLE 98 ANNOYANCE REACTIONS AT PLACE OF WORK, WOMEN, CONT'D

	WOMEN % TOTAL	% WZ	% DZ	WOMEN 26-	36-45	46-58	WOMEN % 26-35	36-45	46-58
HUMIDITY, TOTAL		1752	2 6	34	37		2	700	2
CONC. ANNOYED	1	1.			2 3				.6
VERY ANNOYED	2					9			
DISC.	1	1	1	2	11 1	11	11 9	11	11 1
2		2		2	3 1	2	2 3	3.4	2 0
INTENSE TEMPERATURE CHANGES, TOTAL	42	176	2 C	3	1		2	709	12
CONC. ANNOYED	3.6	4.	3	2	2.4			1	4.
VERY ANNOYED									5
DISC.	1	1.	1		13	1		12	15
2		4.8	1		3		5	3.0	
TOO HOT, TOTAL	21	176	2	33	3		533	49	1255
CONC. ANNOYED	6	4.	3	3.	3.1	5	1	2 0	7
VERY ANNOYED							2		5
DISC.	14.	1.2	5 1	5.	1	11	1 7	16.3	13 5
2									
TOO COLD, TOTAL	2	94	2478	33	494	21	522	94	1253
CONC. ANNOYED	2		1		1	2 1	4	7	3
VERY ANNOYED			3					1	
DISC.	1	1	2	11	1	12	11 5	10.	1 1
2		3.		9	3 1	3	3 3	2 3	5 1
BAD TABLE WORK POSTURES, TOTAL	94	73	2 5	2	7	27	51	6	1246
CONC. ANNOYED		1	3 3		3 3	4	1	1 7	9
VERY ANNOYED					2	1 2	2	1	
DISC.	1 1		1	13	1	13	1	1	17.3
2	3	3.5		3 5	3.8	3	6.	5.4	3

THE ONE TWIN IN THE PAIR STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE

THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

T BLE 89 ANNOYANCE REACTIONS AT PLACE OF WORK, MEN

	MEN			MEN MZ			MEN DZ		
	TOTAL	MZ	DZ	26-35	36-45	46 58	26 35	36-45	46-58
NOISE TOTAL	5 94	2047	3247	463	681	902	731	1032	1 4
CONC ANNOYED	18 9	19 5	18 4	17 7	19 4	20 6	17 1	19 9	18 1
VERY ANNOYED	3 3	3.3	3 3	1 5	2 9	4 5	3 0	1	2 8
1) DISC 1	16.7	14.9	17 8	19 1	14 2	13 9	19 7	18 5	17 6
2) 2	10 2	9 0	11 5	8 6	7 6	7 9	12 6	12 8	10 1
VIBRATIONS TOTAL	5118	1988	3130	442	641	884	704	991	1443
CONC. ANNOYED	3 8	4 6	3 4	4 8	3 9	5 0	2 7	3 9	3 6
VERY AN OYED	4	5	3	2	3	7	3	6	1
DISC. 1	13 8	12 0	15 0	12.7	11 5	12 1	16 1	14 7	1 7
2	4 7	4 0	5 1	3 2	3 8	5	6 1	5 1	4.6
DU T SOOT SMOKE OR GASES TOT L	5229	2024	3 05	449	673	01	721	101	1 65
CONC. ANNOYED	12.2	12 9	11 8	10 2	13 5	13 9	8 9	1 1	11 6
VERY ANNOYED	1 9	2 1	1 8	9	2.4	2 4	1 0	2 7	1 6
DISC. 1	19 4	17 1	20 9	18 5	18 9	15 2	24 7	20.5	1 3
2	9 9	7 8	11 2	8 0	9 4	6 4	11 2	13 1	10 8
ODOR TOT L	5082	1976	3106	440	651	854	701	71	1 7
CONC. ANNOYED	4 5	4.6	4 5	3.9	4 6	5 0	3 4	3 8	5 5
VERY ANNOYED	6	6	6	5	5	5	6	6	7
DISC 1	14 8	12.1	16 3	9 8	13 7	12.2	17 7	17 7	15 1
2	5 0	4.0	5 6	3.9	4 9	3 5	6 1	7 6	8
POOR/GLARING LIGHTING TOTAL	5143	1990	3153	443	664	882	703	999	1 51
CONC. ANNOYED	2 8	3.2	2 6	2 0	2 9	4 0	2 8	2 5	2 6
VERY ANNOYED	3	5	3	5	5	5	1	3	3
DISC. 1	15 6	13 6	16 8	15 8	13 9	12 4	15 8	19 2	15.6
2	4 2	3 8	4 5	3 2	5 4	2 8	3 8	6 4	3

1) THE ONE TWIN IN THE PAIR STATED THAT HE WAS ANNOYED; THE OTHER STATED THAT HE DID NOT NOTICE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT HE WAS VERY ANNOYED; THE OTHER STATED THAT HE DID NOT NOTICE ANYTHING A NOY NG OR THAT HE NOTICED BUT WAS NOT ANNOYED.

TABLE 89 ANNOYANCE REACTIONS AT PLACE OF WORK, MEN CONT D

	MEN			MEN Z			MEN DZ		
	TOT L	MZ	DZ	2 35	36-45	46 58	26 35	36-45	6-59
UM D TY TOTAL	5099	1981	3118	439	663	873	701	981	1494
CONC ANNOYED	1 6	2 0	1 3	1	1	2 6		1 4	1 5
VERY ANNOYED	2	3	1		2	6	1	2	1
) D SC 1	10 2	9.5	11 3	9 3	10.7	6	12 3	13 1	5
2) 2	3 0	2 3	3 4	3 0	2 6	1 8	3 7		2 6
TE SE TEMPERATURE CHANGES TOT L	5158	1993	3165	1	665	85	719	995	1 31
CONC. ANNOYED	5 6	7 1	4 6	5 7	7 2	7 7	3 2	5 2	5.0
VERY ANNOYED	7	1 2	4	5	1 2	1	3	7	3
D SC.	17 1	1 0	18 4	17 5	16 5	12 5	18 6	18.7	18 1
2	7 1	5.9	7 8	5 9	6 9	5 2	8 9	8 7	6 5
TOO HOT TOT L	5115	1 90	3125	443	642	894	712	974	1 35
CONC ANNOYED	3 8	4.2	3 6	2.3	4	5 0	2 7	2	3 6
VERY ANNOYED	7	1 0	5	2	8	1 6	6		6
D SC.	18.2	16.6	19 3	17 6	18 0	15.0	21 3	20 8	17 3
2									
TOO COLD TOTAL	5054	1979	3105	437	661	851	703	970	142
CONC. OYED	3 5	4.2	3 0	3 0	4 5	4 5	2 7	3 9	2 7
VERY OYED	5	8		5	5	1 2		5	2
D SC.	16.8	1 8	18 0	16 5	16 3	12 8	18 6	18 8	17 1
2	5 5	4 5	6 2	1	5 1	2	7 4	2	5 5
UNSUIT BLE WORK POSTURES TOT L	5041	1972	3109	36	636	97	6 7	920	1 32
CONC. AN OYED	7	7 8	7 1		5 3	10 2	2	7 1	8 6
VERY AN OYED		1 1	5	7	1 1	1 3	1	6	6
D SC.	19 1	16.5	20 8	16.3	19 1	1 7	21 2	20	20.
2	7 0	5.7	7 8	5 0	6	5 3	8 3	8 6	7 1

) THE ONE T THE PA ST TED THAT HE ANNOYED; THE OTHER S TED THAT HE D D NOT NOT CE ANY ANNOYANCE

2) THE ONE T THE I ST TED THA HE S VERY ANNOYED; THE OTHE S TED THAT HE D D NOT NOT CE ANYTH NG NOY NG OR THAT HE NOT CED BUT S NOT NOYE

TABLE 39. ANNOYANCE REACTIONS T PLACE OF WORK, WOMEN

	WOMEN TOTAL	WOMEN Z D Z RI	WOMEN DZ	WOMEN Z 26-35	WOMEN Z 36-45	WOMEN Z 46-58	WOMEN C Z 26-35	WOMEN C Z 36-45	WOMEN C Z 46-58
NOISE, TOTAL	4356	1800	2556	544	536		544	721	12 0
CONC. ANNOYED	3 3	4		4.8	4.7	10 5	2 2	3 3	3
VERY ANNOYED		1 1	7	3		1 8	2		
DISC.	1	12 8	14 9	0	14.2	11	1	1 1	1 5
2	1	4.	5	4.1	9	4		1	0
VIBRATIONS, TOTAL	4291	1759	2452		448	33	52	702	123
CONC. ANNOYED		1.2		4	8	1 5		3	
VERY ANNOYED	1	2	1	3	2	1		1	1
DISC.	3 4	4.1	5 8		5 9	3	4	5.1	4.1
2	1 1	1 0	1 2	4			1 8		1
DUST, SOOT SMOKE OR GASES, TOTAL	292	1781	2511	1	2			702	1275
CONC. ANNOYED	3	1.	2.4	1 7	2.4	3	2 1	1 7	3
VERY ANNOYED			3					3	
DISC.	1 1	13.	1	19	3 2	1	1 1	14.	1
2		4.		4.	4.3		9	5	2
ODOR, TOTAL	44	1 5	2455	338	435	13	533	94	126
CONC. ANNOYED	2 8	3.2	2		2 1		2 3	1.	3.2
VERY ANNOYED	3	5			2			1	
DISC. 1	11	10.7	12	1	3	0 2	12	1 1	12
2	2.	2.5	2	8	1	2			2
POOR/GLARING LIGHTING, TOTAL	7	1773	28	341	95	1	53	84	27
CONC. ANNOYED	3	3.	2 1	2 5	1.4		1 1	2.	2
VERY ANNOYED	2	3	2		2			1	2
DISC. 1	12	11.3	13 7	1 7	11	13	13 5	13.1	1 1
2	2	2 2	2 7		3	8	2 1	2.8	2 8

1) THE ONE TWIN IN THE PA STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE.
2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING ANNOYING OR THAT SHE NOTICED BUT WAS NOT ANNOYED

TABLE 39 ANNOYANCE REACTIONS PLACE OF WORK WOMEN CONT'D

	WOMEN TOTAL	WOMEN Z D RI	WOMEN DZ	WOMEN 26-35	WOMEN 36-45	WOMEN 46-	WOMEN 26-35	WOMEN 36-45	WOMEN 46-58
ANXIETY TOTAL	1	732	2 45	40	37			00	1 0
CONC. ANNOYED	1	1.5	8		2				
VERY ANNOYED				3					1
DISC.		1 5	1	2 1	11 1	11	1	1	11 1
2	2.	2.	8	2 5	3 1	2	2 3	3	2 0
INTENSE TEMPERATURE CHANGES TOTAL	4244	76	2500	3 2	4 1		32	05	2 7
CONC. ANNOYED	3		2	2	2			1.	
VERY ANNOYED									
DISC.		1.			1	13	1	12	5
	1	4.	1	4.	3	3	4	3.0	
TOO HOT TOTAL	2	54	24	3	83	2	3	44	12
CONC. ANNOYED		4.		3	3.1	5	1	2	
VERY ANNOYED			3				2		5
DISC. 2	14.	13.	18 1	5 5	1	1	1	1	13
TOO COLD, TOTAL	11	1751	24	83	84	21	532	494	223
CONC. ANNOYED					1	5	4		3.
VERY ANNOYED								1	
DISC.	2	1	12	11	11.	12	11	10	1 1
		4.			3 1	3	3 1	2 8	1
UNSUITABLE WORK POSTURES TOTAL	4254		24			27	17	07	1 44
CONC. ANNOYED	4.	4.	3 3	2	3.1	4	1	1 7	5 0
VERY ANNOYED	3				2	1 1	2		1
DISC. 2	4.	4.1	1	1	4	13	1 3	1	17 3
	4.	3.5		0	3.8	3		3.	3.

THE ONE TWIN IN THE PA STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE
2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING ANNOYING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

TABLE 89 ANNOYANCE REACTIONS AT PLACE OF WORK MEN

	MEN			MEN MZ			MEN DZ		
	TOTAL	MZ	DZ	26-35	36-45	46 58	26 35	36-45	46-58
NOISE TOT L	5 94	2047	3247	443	601	02	731	1032	1 1
CONC. ANNOYED	18 9	19 5	18 4	17 7	19 4	20 6	17 1	19 9	18 1
VERY ANNOYED	3 3	3 3	3 3	1 5	2 9	4 5	3 8	1	2 8
1) DISC. 1	14.7	14 9	17 8	14 1	16 2	13 9	19 7	11 5	17 6
2) 2	10 2	9 0	11 5	8 6	7 6	7 9	12 6	12 8	10.2
VIBRATIONS TOTAL	5118	1988	3130	442	641	884	704	9 1	1445
CONC. ANNOYED	3 8	4 6	3 4	4 8	3.9	5 0	2 7	3 5	3 6
VERY A OYED	4	5	3	2	3	7	3	6	1
DISC. 1	13 8	12 0	15 0	12 7	11 5	12 1	16 1	14 7	1 7
2	4 7	4 0	5 1	3.2	3 8	4 5	6 1	5.1	4.6
DUST SOOT SMOKE OR GASES TOTAL	5229	2024	3 05	449	673	901	721	101	1445
CONC. ANNOYED	12 2	12.9	11 8	10 2	13 5	13 9	8 9	14 1	11 6
VERY ANNOYED	1 9	2.1	1 8	9	2 4	2 4	1 0	2 7	1 6
D SC. 1	19.4	17 1	20 9	18 5	18 9	15 2	24 7	20.3	19 3
2	9 9	7 8	11 2	8.0	9 4	6 4	11 1	13 1	10 8
ODOR TOTAL	5082	1976	3106	440	651	885	701	971	1 34
CONC. ANNOYED	4 5	4 6	4 5	3 9	4 6	5 0	3 6	3.8	9 5
VERY ANNOYED	6	6	6	5	5	5	6	4	7
DISC. 1	14 8	12 1	16 5	9 8	13.7	12 2	17 7	17 7	15.1
2	5 0	4 0	5 6	3 9	4 9	3 5	6 1	7 6	8
POOR/GLARING LIGHTING TOTAL	5143	1990	3153	443	664	882	703	999	1 51
CONC. ANNOYED	2 8	3.2	2 6	2 0	2 9	4 0	2 8	2 5	2 6
VERY ANNOYED	3	5	3	5	5	5	1	3	3
D SC. 1	15 6	13 6	16 8	15 8	13 9	12 4	13 8	19 2	15.6
2	4 2	3 8	4 5	3 2	3 4	2 9	3 8	6 4	3.

1) THE ONE TW N N THE PAIR STATED THAT HE WAS ANNOYED; THE OTHER STATED THAT HE D D NOT NOT CE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT HE WAS VERY ANNOYED; THE OTHER ST TED THAT HE D D NOT NOTICE ANYTHING A OYING OR THAT HE NOTICED BUT WAS NOT ANNOYED

TABLE 89 ANNOYANCE REACTIONS T PLACE OF WORK MEN CONT'D

	MEN			MEN MZ			MEN DZ		
	TOT L	MZ	DZ	26 35	36-45	46-58	26 35	36-45	46-58
HU DITY TOTAL	5099	1981	3118	439	643	878	701	981	1
CON ANNOYED	1 6	2 0	1 3	1.	1 4	2 6	9	2 6	1 5
VERY ANNOYED	2	3	1		2	6	1	2	1
) SC 1	10 2	9 5	11 3	9 3	10 7	6	12 3	13 1	9 5
2) 2	5 0	2 3	3 4	3 0	2 6	1 8	3 7	4.	2 6
NTENSE TEMPER TURE CHANGES TOTAL	5158	1 93	3165	1	665	896	719	995	1 51
CONC A OYED	5 6	7 1	4 6	5 7	7.2	7	1 2	9 2	5 0
VERY ANNOYED	7	1 2	4	5	1 2	1	3	7	2
D SC 1	17 1	19 0	18 4	17 5	16 5	12 9	18 6	18 7	18 1
2	7 1	9.9	7 8	5 9	6 9	5 2	8 9	8 7	4 5
TOO OT TOTAL	5119	1990	3125	443	642	854	712	979	1 15
CONC. ANNOYED	3 8	2	3 6	2 3		5 8	2 7	2	3 8
VERY ANNOYED	7	1 0	5	2	8	1 6	6	4	4
D SC	18.2	16.	19 3	17 6	18 0	19 0	21 3	20 8	17 3
2									
TOO COLD TOTAL	5054	1979	3105	37	661	891	703	978	1 1
CONC. OYED	3 9	4 2	3 0	3 0	4 5	4 5	2 7	3 8	3 7
VERY OYED	5	8		5	9	1 2		9	2
D SC	16.8	14.8	18 0	14.5	1 3	12 8	18 6	18 8	17 1
2	5 5	4 5	2	1	3 1	2	7	7	5 5
UNSU TABLE ORK POSTURES TOTAL	5081	1972	3109	36	636	9	647	980	1 33
CONC. ANNOYED		7 8	7 1	8.	5 8	10 2	2	7 1	8 6
VERY ANNOYED	7	1 5	5	7	1 1	1 3	1	6	
D SC. 1	19 1	14 5	20 8	1 3	19 1	1 7	21 2	20 7	20.6
2	7 0	9.7	7 8	9 8	6	5 8	8 3	8	7 1

1) THE ONE T THE A S TED HA HE A OYED THE OTHER T TED THAT HE D D NOT NOTICE ANY ANNOYANCE

2) THE ONE THE A STATED THA HE S VERY ANNOYED THE OTHE ST TED THAT HE D D NOT T CE ANYT NG OYI G OR THAT HE NOT CED BUT NOT ANNOY

TABLE [illegible]. ANNOYANCE REACTIONS AT PLACE OF RESIDENCE, WOMEN.

	WOMEN TOTAL	NZ	DK	WOMEN I 26–35	36–45	46–58	WOMEN 2 26–35	36–45	46–58
TRAFFIC NOISE, TOTAL	654	2547	4009	5 6	851	1199	1010	1253	17 3
CONC. ANNOYED	2 2	2 6	1 9	2.1	1 3	3.5	1 5	1	2
VERY ANNOYED	7 4	6 0	8	16 7	6.	1	10 / 3.0	3.2	3
1) DISC.	2.8	1.3	3 0	3.9	2 1				
AIRCRAFT NOISE, TOTAL	551	240	3943	5 9	840	119	73	1238	1711
CONC. ANNOYED	1 8	1.	8	5	7	2 0	6		9
VERY ANNOYED	7	1.9	5 2	8	.5	3 2	5	8	7
DISC.	1 1	1 1	1 1	1 9	6		1	1 1	1 1
INDUSTRIAL NOISE	6490	2547	3913	565	827	1173	974	122	1709
CONC. ANNOYED	2	2	2			1	2 3	2 1	1
VERY ANNOYED	2 1	2.1	2 0	2 7	2 3	1			
DISC.	3	3	5	7	7	2			
NOISE FROM NEIGHBOURS, TOTAL	595	2 20	3975	593	846	11 2	1031	1244	1727
CONC. ANNOYED	3.1	3.	2	1 2	3.1		7	1	4.
VERY ANNOYED	3	3	3	3	1		1	8 5	
DISC.	9 1	6.3	4	1	1 3	1	2	2	2 5
	2 1	1 7	2						
ODOUR, TOTAL	6545	2601	39	5 5	837	11	1	1237	1713
CONC. ANNOYED	2	2.	2	2 1	1.	3	1.6	1	3.
VERY ANNOYED	2		2	14.1	12.	6.3	13 5	12 8	9 9
DISC.	1 2		11 5	4.0	2	1 3	2 1	3.1	2 5
	2 5	2 1	2 6						

1) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE.

2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING ANNOYING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

TABLE [illegible]. ANNOYANCE REACTIONS AT PLACE OF RESIDENCE, WOMEN, CONT'D.

	WOMEN TOTAL	NZ	DK	WOMEN I 26–35	36–45	46–58	WOMEN 2 26–35	36–45	46–58
AUTOMOBILE EXHAUST/GASES, TOTAL	3 3	10	3	588		1197		13	1730
CONC. ANNOYED		2.	2 2	1 6	1.2		1	9	7.6
VERY ANNOYED	3			5	2				9
1) DISC.	19.	6.7	1	1	8		1 8	10	11
2)	2		3	1	2	1	4.	2	
AIR POLLUTION, TOTAL	652	258	37	72	829	11	56	123	1 1
CONC. ANNOYED	2	2.8		1 6	1	4.	1	1 1	3
VERY ANNOYED				8				1	
DISC.	11		11	1 7	1	0	1	18	1 0
	3 1		3 5	3.1	2.	1	4.	3 0	3.6
DUST, SOOT, TOTAL	6543	2549	39 5	5	5	119	8	12 1	1 1
CONC. ANNOYED	3 1	2.1		1.6	.2	1	1	2 7	8
VERY ANNOYED		6	1	2		8	1	3	
DISC.	13 5	11 8	1	15 0	1 1		16 8	19	13
	6.	3 8	0	3.	4.2	2 2	6.0		6
WATER POLLUTION, TOTAL	66 2	958	3594	567	819	1170	9 9	1227	1785
CONC. ANNOYED	8	1	5	1 7	3	1	1	1	1 8
VERY ANNOYED	1	2	1			9	1		1
DISC.		5.	.0	6 2	5	5 2	7 8	9 7	7 9
	2.	2.8	2 1	2 9	1.8	3 3	2 8	1	7

1) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANY ANNOYANCE.

2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED; THE OTHER STATED THAT SHE DID NOT NOTICE ANYTHING ANNOYING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

TABLE 810 ANNOYANCE REACTIONS AT PLACE OF RESIDENCE MEN

	MEN TOTAL	MZ	DZ	MEN MZ 26-35	36-45	46-55	MEN DZ 26-35	36-45	46-55
TRAFFIC NOISE TOTAL	5815	2232	3583	501	703	1027	793	1083	1707
CONC. ANNOYED	2 5	3.0	2 2	3.0	2 6	3 4	1 5	1.4	2 9
VERY ANNOYED	2	3	2		3	4	3	2	2
1) DISC 1	8.7	8 3	9 0	10 3	11 0	5	11 7	9 3	7 3
2) 2	2 8	2 6	2 9	2 4	3 0	2 3	4 5	2 9	2 2
AIRCRAFT NOISE TOTAL	5729	2198	3531	488	696	1013	773	1066	1692
CONC. ANNOYED	1 3	1 4	1 3	8	1 3	1 8	1 3	9	1 5
VERY ANNOYED	2	2	3	2		4	3		2
DISC. 1	5 5	5 5	5 6	8 6	4 9	6 6	6 6	5 9	6 9
2	1 1	1 0	1 2	2 0	7	7	2 3	8	1 0
INDUSTRIAL NOISE	5673	2171	3502	481	691	998	772	1035	1675
CONC ANNOYED	5	6	4	2	3	9	6	1	1
VERY ANNOYED	1	1				2	1		
DISC. 1	3 0	2.4	3 4	3 1	2 3	2 1	3 2	2	1 5
2	9	6	1 1	2	9	7	1 2	1 0	1.1
NOISE FROM NEIGHBORS TOTAL	5748	2202	3556	490	701	1010	787	1073	1697
CONC ANNOYED	1 8	2 3	1 6	1 6	2 0	2 8	5	1.0	2.
VERY ANNOYED	1	1	1			3			3
DISC 1	8 1	7 8	8 2	8 4	9 8	6 1	7 8	9 6	7 5
2	1 9	2 2	1.8	2 4	2 9	1 6	1 3	2 4	1 6
ODOR TOTAL	5723	2191	3532	487	693	1010	785	1066	1681
CONC. ANNOYED	1 9	2 5	1 6	1 6	1 5	3.7	1 1	1 2	2 8
VERY ANNOYED	2	4	1			6			2
DISC.	10.0	9 3	10 4	10 9	11 5	6 9	11 2	11 2	9.6
2	2 6	2 6	2 3	3 1	3 0	2 2	2 7	2 3	2 8

1) THE ONE TWIN IN THE PAIR STATED THAT HE WAS ANNOYED; THE OTHER STATED THAT HE DID NOT NOTICE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT HE WAS VERY ANNOYED; THE OTHER STATED THAT HE DID NOT NOTICE ANYTHING ANNOYING OR THAT HE NOTICED BUT AS NOT ANNOYED.

TABL 810 ANNOYANCE REACTIONS AT PLACE OF RESIDENCE MEN CONT'D

	MEN TOTAL	MZ	DZ	MEN MZ 26-35	36-45	46-55	MEN DZ 26-35	36-45	46-55
AUTOMOBILE EXHAUST GASES TOTAL	5734	2193	3541	489	692	1013	783	1068	1690
CONC ANNOYED	1 5	1 8	1 3	1 0	9	2	9	7	1 1
VERY ANNOYED	2	4	3		1	7	3		1
1) DISC 1	8 5	7 6	9 1	8 0	8 5	6 7	9 9	8.9	9 1
2) 2	2 4	2 0	2 7	3 5	1.4	1 7	3 2	2 1	2 9
AIR POLLUTION TOTAL	5722	2193	3529	489	692	1011	776	1066	1689
CONC. ANNOYED	2 0	2.2	1 8	1 0	1 7	3 2	1 0	1	2
VERY ANNOYED	3	3	3		1	4	3	2	4
DISC 1	10 3	9 8	10 3	9 6	11 0	9 9	11 3	10 2	9 9
2	2 7	2 7	2 7	3 5	2 7	2	2 8	2	2 3
DUST SOOT TOTAL	5722	2190	3532	87	694	1009	780	10 5	1687
CONC ANNOYED	1 6	1 8	1 5	1 2	1 9	2 1	9	1 7	1 6
VERY ANNOYED	2	2	3			4			1
DISC. 1	10 8	9 8	11 4	10.7	12 5	7 5	10 7	12	9 7
2	3 1	2 7	3.4	2 7	3 6	2 1	3 5	3	3
WATER POLLUTION TOTAL	5666	2179	3 85	48	689	1005	772	1051	1662
CONC. ANNOYED	5	5	3	4		5	4	3	8
VERY ANNOYED	1		1			1	1	1	2
DISC 1	6.	4.1	6 6	6 8	6 0	6 3	5 8	6	7
2	2 1	2 2	2 1	2 3	1 9	2 3	1 5	2 6	2 1

1 THE ONE TWI THE PA R ST TED THA HE S ANNOYED; THE OTHER ST TED THA HE D D NOT NOT CE ANY ANNOY NCE

2) THE ONE TWIN IN THE PA R ST TED THAT HE WAS VERY ANNOYED THE OTHE ST TED THAT HE D D NOT NOT CE ANYTH NG NNOY NG OR THAT HE NOT CED BUT S NOT ANNOYED.

TABLE 8A. ANNOYANCE REACTIONS AT PLACE OF RESIDENCE, WOMEN.

	WOMEN	Z DZ		WOMEN	Z		WOMEN	DZ	
	TOTAL	MZ	DZ	26-35	36-45	46	26-35	36-4	46-55
TRAFFIC NOISE, TOTAL	5	244	4004	5 6	831	1194	151	1253	17 3
DISC, ANNOYED	2 2	2	1 9	7	1 3	3	1 5	1	2.9
VERY ANNOYED	2	1	1		1	7	2		2
D DISC, 1	7 0	6 0	8	10 7	6	9	10		7 2
2	2.	2.3	3 0	3.9	2 1	1 7	5.0	3.2	2 9
AIRCRAFT NOISE, TOTAL	551	240	3948	5 9	840	11	93	1230	1711
DISC, ANNOYED	1 0	1.3	0	8	7	2.0	6	4	
VERY ANNOYED		1				7			
DISC.	4 7	1.9	5 2	0	4.8	3 2	5	3	7
2	1 1	1 1	1 1	1 9	8		1	1 1	1 1
INDUSTRIAL NOISE	6480	2547	3913	865	827	1173	7	122	1709
DISC, ANNOYED	2	2	2					1	
VERY ANNOYED						1			1
DISC.	2 2	2.1	2 0	2 7	2	1	2 3	2 1	1 9
2	5	5	5	7	7		3		
NOISE FROM NEIGHBORS, TOTAL	993	2 20	3978	592	846	11 2	1001	1244	1727
DISC, ANNOYED	3 1	1.	2 7	1 2	2.1	4 0	7	1	
VERY ANNOYED	3	2	3						
DISC.	9 1	4.3	6	3	1	7	10	8 5	9
3	2.1	1 7	2.4	9	1 3	1	2 4	3 2	2 5
BOP, TOTAL	94	2401	3944	9 5	87	1 57	1	1237	1 11
DISC, ANNOYED	2 6	2.7	2	2 1	1 9		1	3	3.9
VERY ANNOYED	2	3	2	2				1	3
DISC.	11	0.9	11 5	1 1	12 5	8 3	13 5	2.0	
2	2.	3	2	4.	2 7	1	2 1	3.1	2

1) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS ANNOYED, THE OTHER STATED THA SHE DID NOT NOT CE ANY ANNOYANCE

2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED, THE OTHE STATED THAT SHE D NOT NOTICE ANYTHING ANNOYING OR THAT SHE NOTICED BUT WAS NOT ANNOYED.

TABLE 8A. ANNOYANCE REACTIONS AT PLACE OF RESIDENCE, WOMEN CONT'D.

	WOMEN	D Z		WOMEN			WOMEN	Z	
	TOTAL	MZ	DZ	26-35	36-4	46-58	26-35	36-4	46-5
AUTOMOBILE EXHAUST/FUMES TOTAL	9	2 0	39	80	8	11	97	12 3	1 80
DISC, ANNOYED	2		2	1	1 2		1	9	7
VERY ANNOYED	3		2				1		9
DISC, 1	10.	8.	11	11	8		1 0	1	11
2	3.	3		1	2 1	1		2	
AIR POLLUTION, TOTAL	529	258	39 1	9 7	829	11	94	123	17 8
DISC, ANNOYED	2.	2.	2	1	2		1 9	1 1	3.4
VERY ANNOYED								1	
DISC.	11		12	11	12		1 1	17	11 0
	1	2.	3 8	3.1	2	1		3 0	3
SOOT, DUST, TOTAL	4543	259	39 3		833	11	9	12 1	1717
DISC, ANNOYED	3 1	2.	3 0	1 6	3.2	1	1 7	2	0
VERY ANNOYED		9		2	2		1	2	
DISC. 2	13 8	11	1 .4	19 8	1	0	1 0	15	13 0
	4.	3 5	0	3.	2	2 2	0		
WATER POLLUTION, TOTAL	6492	8	3694	967	81	1170	95	1227	1 85
DISC, ANNOYED	0	1 2		1 2	9	1 6	1	1	1 0
VERY ANNOYED	1	2	1			4	3		2
DISC. 2		5.	0	6 2	9	9 3	7 0	5	7
	3	2.0	2 1	2 3	1	2 3	2 0	1	2 6

1) THE ONE TWIN IN THE PAI STATED THAT SHE WAS ANNOYED, THE OTHER STATED THA SHE DI NOT NOTICE ANY ANNOYANCE,

2) THE ONE TWIN IN THE PAIR STATED THAT SHE WAS VERY ANNOYED, THE OTHER ST TED THAT SHE DID NOT NOTICE ANYT ING ANNOYING OR THA SHE NOTICED BUT WAS NOT ANNOYED.

TABLE B11 ANNOYANCE REACTIONS GENERAL MEASUREMENTS MEN

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 26–35	MEN MZ 36–45	MEN MZ 46–58	MEN DZ 26–35	MEN DZ 36–45	MEN DZ 46–58
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT TOTAL	5946	2293	3703	516	718	1058	823	1116	1764
1) CONC. ANNOYED	12 9	14 6	11 9	12 6	12 5	16 9	9 5	11 4	13.3
NOT ANNOYED	54 3	55 5	53 6	51 7	51 8	59 7	53 6	51 8	54 5
DISCORDANCE	34 8	30 0	34 6	35 7	35 7	23 3	36.9	36.8	32 0
1) CONC. VERY ANNOYED	1 5	1 6	1 5	4	8	2 6	1 1	1 0	1 9
NOT ANNOYED	86 8	86 7	86 9	85 9	86 9	87 1	85 8	86.9	87 4
DISCORDANCE	11 7	11 7	11 6	13 6	12 3	10 3	13 1	12 1	10.7
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE TOTAL	5096	2293	3703	516	718	1059	823	1116	1764
DISC. 2 VRS 5–	1 4	1 4	1 4	1 6	1 3	1 3	1 7	1 2	1.3
ANNOYED AT PLACE OF WORK BY ANY AGENT TOTAL	5996	2293	3703	516	718	1058	823	1116	1764
4) CONC. ANNOYED	33 4	34 8	32 5	32 2	37 9	33 9	32 0	33 7	31 7
NOT ANNOYED	28 6	30 4	27 4	28 9	24 9	34 9	26 5	21 1	31 8
DISCORDANCE	38 1	34 8	40 0	39 0	37 2	31 2	41 5	3.2	36 5
4) CONC. VERY ANNOYED	7 1	7 6	6 8	5 2	8 5	8 1	6 1	8 5	6.0
NOT ANNOYED	65 4	67 8	63 9	70.7	62 7	6 8	61 7	57 8	61 8
DISCORDANCE	27 5	24 6	29 3	24 0	28 8	22 0	32 2	33.7	25.2
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK, TOTAL	5996	2293	3703	516	718	1058	823	1116	1764
DISC. 2 VRS 5–	6 6	5 5	7 3	6 4	6 8	4 3	5 8	8 1	7

1) ANNOYED BY ANY AGENT

TABLE B11 ANNOYANCE REACTIONS GENERAL MEASUREMENTS WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 26–35	WOMEN MZ 36–45	WOMEN MZ 46–58	WOMEN DZ 26–35	WOMEN DZ 36–45	WOMEN DZ 46–58
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT TOTAL	5911	2736	173	11	850	124	1953	1304	1 01
1) CONC. ANNOYED	3 8	15 0	1 7	13 6	14.1	20	11 8	11 1	1 2
NOT ANNOYED	50 4	5	8 8	50 1	51 5	55 2	9 4	50.6	7 7
DISCORDANCE	34.0	30.2	36 5	36.3	34.4	24 1	39	35 3	33 3
1) CONC. VERY ANNOYED	1 9	2 0	1 8	1 0	8		9	9	2 9
NOT ANNOYED	84.9	86.1	85 1	83 0	86 7	87 1	8 2	85 7	87 9
DISCORDANCE	13 2	11 9	14 1	16 0	12 5	9	1 9	13 3	1 5
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE TOTAL	6909	2736	173	611	800	1242	1053	1304	1801
DISC. 2 VRS 5–	1 2	1 1	1 2	1 3	8	1 2	1	8	1 6
ANNOYED AT PLACE OF WORK BY ANY AGENT TOTAL	959	2736	4173	11	883	12 2	13 3	1104	18.3
1) CONC. ANNOYED	15 1	17 7	13.4	10 0	12 6	25	8 7	8	1 5
NOT ANNOYED	50 2	50.5	50 0	53 8	54	4	54 5	5 1	.4
DISCORDANCE	34 7	31 7	36 6	36 2	31 0	30 0	36 9	37 0	36.2
1) CONC. VERY ANNOYED	2	3 3	1 8	2 1	2 3			1 8	3 1
NOT ANNOYED	81	81 9	8 5	82 7	85 2	79 1	84.5	1	5.0
DISCORDANCE	16 0	1 8	1 8	15.2	12 5	16	33 1	16 6	17
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK TOTAL	6909	273	173	11	810	12 7	1383	1304	1803
DISC. 2 VRS 5–	1 8	2 0	1 4	1 3	1 6	2	1 8	8	2 8

1) ANNOYED BY ANY AGENT

TABLE 6I2. EDUCATION, OCCUPATION, AND WORK SITUATION MEN

	MEN			MZ			MEN		
	I DZ			Z			DZ		
	TOTAL	MZ	DZ	26-35	36-45	46-5	26-35	36-45	46-55
EDUCATION, MANDATORY 9 YEARS OR LESS,									
TOTAL	5947	227	3 8	13	71	1 51	15	1113	1740
CONC. MORE	35 0	35.	34 7	42 3	31 0	35 5	37 8	29 0	3
HIGHER EDUCATION	1 9	7 8	1 7	35.3	51 8	2	31 3	40.	0.
DISCORDANCE	22	17 0	26.8	22 2	16.9	14.	30 2	2 6	21 8
PRESENT GAINFUL EMPLOYMENT									
TOTAL	5980	2121	3407	475	687	58	751	1065	1593
CONC. FULL TIME	63 5	64.	61 8	83.	84.	64 5	82 2	83 2	37
PART-TIME		7			1	1	1	1	1 1
NOT GAINFULL EMPLOYED	14 1	14.1	14 2	1 9		34.4	1 3	1 5	32 7
DISC. FULL/PART	2.6	2.	2 8	2 3	2 6	2 7	1 5	2 9	1.3
FULL/NOT	15.	12 7	17 8	13 5	9.5	1 6	1 .4	11 2	22.2
PART/NOT	1 5	1 1	1 8	2	3	2 2		1 1	2 8
CHANGE OF EMPLOYER DURING THE LAST TEN YEARS, TOTAL	466	1822	28 7	441	640	743	87	994	1146
CONC. 4- TIME(S)	3.9	4.	8	1 6	4 7	5	7	3.	5.3
0-3	79 2	80.1	78	89 3	75.4	78	88 8	75 9	7
DISCORDANCE	16.	1 5	17 9	9 3	19 3	15	10 5	20.7	19.
DISC. 1/	1 7	1 7	1 7	9	2.3	1 9	1	2	3.3

TABLE 6I2. EDUCATION, OCCUPATION AND WORK SITUATION MEN CONT'

	MEN			MEN			MZ		
				Z			Z		
	TOTAL	MZ	DZ	26-35	36-45	46-56	26-35	36-45	46-54
SHIFT-TIME, AT PRESENT									
TOTAL	54 5	7 30	8323		7	10	777	1079	1 71
CONC. YES	1	3.3	1 0	1	6	7	15 1	18 3	8.4
NO	59 1	1	57	2	51	7	44.5	9.3	72 5
DISCORDANCE	29 1	29	31	33 2	31 4	1	38 9	30	21 3
EXTRA JOB NOW OR EARLIER									
TOTAL	542	2988	3332		402	996	770	106	1 95
CONC. YES	2	6.8		4.	7.4	11 3	5	8	7
NO	66.6	3	64 1	64 0	45.6	45.4	6 9	3.6	6
DISCORDANCE	16.	4.2	27 3	2 3	37 0	19	24 5	1	2 2
SHIFT-WORK NOW OR EARLIER,									
TOTAL	5400	2 1	3 5	803	69	1	775	1080	151
CONC. YES	5	10.	7	10.0	11.	10 2		10.1	7 7
NO	7 1	9.1	66.5	3	66.0	73 2	62 3	9 6	7 0
DISCORDANCE	23.	21.2	34	26	21 3	1	28.	38.9	14.
PIECEWORK NOW OR EARLIER									
TOTAL	94	21 5	3368	44	780	9	770	1094	1506
CONC. YES	3 3	25.4	21	26	2 9	21 1	21 1	21	1 .4
NO	43	49.1	3 7	44.	1.4	56.3	43.	33	5 7
DISCORDANCE	3 8	25.3	34.4	26.	29 7	20	3	39.0	2 .0
UNEMPLOYED NOW OR EARLIER									
TOTAL	5472	2113	3359	497	787	58	8	1098	1491
CONC. YES	1.	4.	3.6		2 7	3	1.2	1.4	2
NO	86.		7	86.1	8	7 .8	88 3	88	7 6
DISCORDANCE	14.2	14.1	17.6	11.3	13.4	1 0	10 4	1 .8	21 1

TABLE B11 ANNOYANCE REACTIONS GENERAL MEASUREMENTS MEN

	MEN TOTAL	MEN MZ	MEN DZ	MEN MZ 24-35	MEN MZ 36-45	MEN MZ 46-58	MEN DZ 24-35	MEN DZ 36-45	MEN DZ 46-59
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT TOTAL	59 6	2293	3703	516	718	1058	823	1116	1744
1) CONC. ANNOYED	12 9	14 6	11 9	12 6	12 5	16 9	9 5	11 4	13.3
NOT ANNOYED	54 3	55 5	53 6	51 7	51 8	59 7	53 6	51 6	54.6
DISCORDANCE	3- 8	30 0	34 6	35 7	35 7	23 3	36 9	36 8	32 0
1) CONC. VERY ANNOYED	1 5	1 6	1 5	4	3	2 6	2 2	1 0	1 9
NOT ANNOYED	86 8	86 7	86 9	85 9	86 9	87 1	85 8	86 9	87 4
DISCORDANCE	11 7	11 7	11 6	13 8	12 3	10 3	13 1	12 1	10 7
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE TOTAL	5996	2293	3703	516	718	1058	823	1116	1744
DISC. -2 YRS 5-	1 4	1 4	1 4	1 6	1 3	1 9	1 7	1 2	1 3
ANNOYED AT PLACE OF WORK BY ANY AGENT TOTAL	5996	2293	3703	516	718	1058	823	1116	1744
1) CONC. ANNOYED	33 4	34 8	32 5	32 2	37 9	33 9	30 0	35 7	31 7
NOT ANNOYED	28 6	30 4	27 4	28 9	26 9	34 9	26 3	21 1	31 8
DISCORDANCE	38 1	34 8	40 0	39 0	37 2	31 2	43 5	3.2	3 5
1) CONC. VERY ANNOYED	7 1	7 6	6 8	5 2	8 5	8 1	6 1	8 5	6.0
NOT ANNOYED	65 4	67 8	63 9	70.7	62 7	6 8	61 7	57 8	68 8
DISCORDANCE	27 5	24 6	23 3	24 0	28 8	22 0	32 2	33.7	25.2
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK, TOTAL	5996	2293	3703	516	718	1058	823	1116	1744
DISC. 2 YRS 5-	4 6	5 5	7 3	4 4	4.8	4 8	5 8	8 1	7

1) ANNOYED BY ANY AGENT

TABLE B 1 ANNOYANCE REACTIONS GENERAL MEASUREMENTS WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 24-35	WOMEN MZ 36-45	WOMEN MZ 46-58	WOMEN DZ 24-35	WOMEN DZ 36-45	WOMEN DZ 46-58
ANNOYED AT PLACE OF RESIDENCE BY ANY AGENT TOTAL	39	2 36	1 3	11	830	12	1953	1304	1 03
1) CONC. ANNOYED	1 4	1 0	14 7	13 6	14.1	20	11 8	11 1	1 3
NOT ANNOYED	50 4	5	8 8	50 1	51 5	55 2	9	50 6	47 7
DISCORDANCE	34 0	30 2	36 5	36.3	34 4	2 1	39	38 3	33 3
1) CONC. VERY ANNOYED	1 9	2 0	1 8	2 0	8		9	9	2 9
NOT ANNOYED	84 9	84 1	84 1	83 0	84 7	87 1	84 2	85 7	82 9
DISCORDANCE	13 2	11 9	14 1	16 0	12 5		14 9	13 3	1 1
NUMBER OF ANNOYING AGENTS AT PLACE OF RESIDENCE, TOTAL	690	2 16	173	611	880	1242	1063	1304	1883
DISC. 2 YRS 5-	1 2	1 1	1 2	1 3	8	1 2	1	6	1 6
ANNOYED AT PLACE OF WORK BY ANY AGENT TOTAL	939	273	4173	11	883	12 7	13 3	1304	1403
1) CONC. ANNOYED	15 1	17 7	11 4	10 0	12	25	9 7		1 9
NOT ANNOYED	50 2	40.5	50 0	53.8	56 4		54 5	54.1	
DISCORDANCE	34 7	31 7	36 6	36 2	31 0	30	36 9	37 0	4.1
1) CONC. VERY ANNOYED	2	3 3	1 8	2 1	2 3			1 0	3 1
NOT ANNOYED	81	81 9	8 5	92 7	85 2	7 1	8 5	2	79 0
DISCORDANCE	16 0	1 8	1 8	15 2	12 5	16 3	15 1	16 6	17 9
NUMBER OF ANNOYING AGENTS AT PLACE OF WORK TOTAL	4909	273	4173	611	810	12 7	2363	1304	1807
DISC. 2 YRS 5-	1 8	2 0	1 4	1 3	1	8	1 0	8	2 6

1) ANNOYED BY ANY AGENT

TABLE 811.

EDUCATION, OCCUPATION AND WORK SITUATION MEN

	MEN TOTAL	MEN Z MZ	MEN D Z OZ	MEN Z 24 35	MEN Z 36-4	MEN Z 46-58	MEN Z 26-35	MEN Z 36- 5	MEN Z 6-59
EDUCATION: MANDATORY 9 YEARS OR LESS									
TOTAL	5947	2279	3648	13	71	1051	818	1113	1740
CONC. ABOVE	35 0	35.	34	42 3	31 8	5	17 8	29 0	34
HIGHER EDUCATION	1 9	0	3 7	5	81 8	49.2	31 3	40.	6.
DISCORDANCE	22 6	17 0	24.8	22 2	24.9	1	10 2	29	1
PRESENT GAINFUL EMPLOYMENT									
TOTAL	5530	2121	3 C9	73	487	38	751	1949	1593
CONC. FULL-TIME	8 6	64.9	1.8	83	84.	44 5	82 2	83.2	27 4
PART-TIME		7			1	1	1	1	1 1
NOT GAINFULLY EMPLOYED	1 1	16.1	1 1	1 3		1	1 5	1 9	12 7
DISC. FULL/PART	2 6	8	2.8	1 3	2.6	2 7	1 8	2.9	8 8
FULL/NOT	15.	12 7	17	13.3	9.5	1 .6	16 4	11 2	22.2
PART/NOT	1 8	1 1	1 8	2	3	2 2		1 1	2 6
CHANGE OF EMPLOYER DURING THE LAST SIX YEARS, TOTAL	464	1422	38 7	441	640	7 3	87	994	1144
CONC. 4- TIMES)	2.	4.	3 5	1	7	8	7	3.	9.3
0-8	79.2	80.1	78	89.3	75.	78	88	73 9	7 8
DISCORDANCE	16.	18 8	17	3	19 5	15	10.8	20.7	19.
DISC. 1/	1 7	1 7	1 7	8	2.3	1	1	2.8	2.3

TABLE 812.

EDUCATION OCCUPATION AND WORK TUATION, MEN CONT'

	MEN TOTAL	MEN Z MZ	MEN O OZ	MEN 24 35	MEN 36-45	MEN 46-48	MEN Z 26-23	MEN Z 36-4	MEN Z 46-5
OWN-TIME, AT PRESENT									
TOTAL	54 6	7*2	3323		7	10	777	1879	71
CONC. YES		8.3	11 0	1	16.8		15 1	18	5
NO	39	1	57	2	81	7		5 3	72 5
DISCORDANCE	29	21	11 3	12.2	3	1	38 5	3*	21 5
EXTRA JOB NOW OR EARL ER,									
TOTAL	542	208	32		492	96	776	10	1 95
CONC. YES	2	5		4.	7	11	3	8	
NO	44	3	46	66	3.6	49	4 9	3	6
DISCORDANCE	26.1	4.2	2 8	29.3	2		24 5	11.6	24.2
SHIFT-WORK NOW OR EARLIER,									
TOTAL	3488	18	33	8 3	69	1	775	1080	1528
CONC. YES	5	8.		18.8	1 1.			16.1	7 7
NO	1	8.	6.5	3	64.	3.2	62 3	9	7
DISCORDANCE	23	21 2	24	26	23 3	1	23.	18.	18.3
PIECEWORK NOW OR EARLIER									
TOTAL	84 3	115	3368	49	700	19	778	108	1506
CONC. YES	3.3	5.	1	24	28	23 1	2 1	27	1
NO	48.8	4.1	7	44.	2.	56 3	3	3.	12
DISCORDANCE	1	23.5	36	2	29	2	34.6	39.	2 .8
UNEMPLOYED NOW OR EARL ER,									
TOTAL	8472	3213	33	47	707	954	48	1008	1 91
CONC. YES	2.	4.	2		2.	.3	1.2	1.4	.2
NO	84.	1	79	89 1	61.	7*.8	88 5	8.	7
DISCORDANCE	14.	14.1	1	11.9	18	1	10	18 8	21 1

TABLE B12 EDUCATION OCCUPATION AND WORK SITUATION WOMEN

	WOMEN MZ DZ TOTAL	MZ	DZ	WOMEN MZ 26 35	36-45	46 58	WOM DZ 26-35	36- 5	6-55
EDUCATION MANDATORY 9 YEARS OR LESS									
TOTAL	64	2713	142	404	874	1213	1312	1196	1793
CONC, ABOVE	38 0	37 6	33 2	46 2	33 1	34 5	9 1	33 4	15 1
HIGHER EDUCATION	2 1	44.9	3 C	37 0	47 5	51	2 2	1 4	7
DISCORDANCE	17 0	14 3	2 9	16 2	18 1	11 1	25 7	23 8	12 3
PRESENT GAINFUL EMPLOYMENT									
TOTAL	6444	2 49	897	578	838	1153	86	123	188
CONC, FULL TIME	1 3	71 4	17 4	1 4	17 4	27	12 2	19	32.2
PART TIME	9 2	1	7 6	17 8	11 5	3 3	13 7	4	7 1
NOT GAINFULLY EMPLOYED	5 5	24 9	2 6	13 3	19 0	39	13 1	1 1	1 6
DISC FULL/PART	14 0	12 9	14 7	1 6	14 1	7 1	24 4	15 7	6 6
FULL/NOT	19 7	18 4	20 7	1 0	19 3	18	16 5	20 2	23 3
PART/NOT	23	11 2	15 0	17 0	14 7	4 3	20 2	20	7 9
CHANGE OF EMPLOYER DURING THE LAST									
TEN YEARS TOTAL	4894	1958	2 36	409	679	8	709	99	1194
CONC, 4- TIME(S)	2 1	1 3	1 9	2	1 3	4		7	0
0-3	8 0	85.6	84 7	96 8	84 8	80	96 5	85 5	77 3
DISCORDANCE	12 9	12 1	13 4	2 9	13 8	15 0	3 5	13 9	15 1
DISC 3/4	1 6	1 8	1 5		1 9	2 6	1	1 7	2 C

TABLE B12 EDUCAT ON OCCUPAT ON AND WORK SITUATIO WOMEN CONT D

	WOMEN Z DZ TOTAL	MZ	DZ	WOME Z 26 35	3 -45	5	WOMEN DZ 26-35	36 45	5-55
OVER-TIME AT PRESENT									
TOTAL	53	7150	12 0	4+2		10	7 9	87	144
CONC YES	1		1 1		9	2	1	1	
NO	8	8 8	85 1	4	86 0	9A ?	83 0	A4	
DISCORDANCE	13 0	11 8	13 8	13 0	11 5	11	19 8	14 0	12 3
EXTRA JOB NOW OR EARL ER									
TOTAL	7 2	18	3 64	9 0	7 0	1	9 1	11	1 1
CONC YES	G	5 0	3 3	1	2 0	5		1 0	1
NO	80 8	40 7	83 8	87	8C 5	7	8 1	2	7 7
DISCORDANCE	19 2	14.3	19 8	11 2	17	13	13 3	1 5	1 7
SHIFT-WORK NOW OR EARL ER									
TOTAL	c	2331	3 72	502	7 9	1	5	1 08	19
CONC, YES	8	1 3	2 5		2			2 0	1 2
NO	83 3	9 5	81 9	6 3	83 8	87	82 5	8.	2 3
D SCORDANCE	13 8	11 2	15	1 3	13	8	15	17 1	1
P ECEWORK NOW OR EARLIER									
TOT L	581	347	3 70	11	3.6	13	9 2	111	1 19
CONC, YES	2	5	4 0	5	7 7		5 3		
NO	7	1 1	7C 5	7 3	78 0	5	3 7	7	87 0
D SCORDANCE	1 2 1		15 5	1 4	1 3		23 0	19	1 3
UNEMPLOYED NOW OR EARL ER									
TOTAL	5 35	2 0	3 01	993	7 3	10		104	1532
CONC, YES	2	3.1	2 7		1 5		7	5	
NO	8	8	8 2	8 7	8	91	1 0	88 8	
D SCORDANCE	12	11 3	13 1	7	4 3	1	8 3	11 9	17 0

TABLE 8/1. RESIDENCE SITUATION, SENSE OF WELL-BEING, ETC. MEN

	MEN % TOTAL	NZ	OZ	MEN % 26-35	36-45	46-5	MEN OZ 26-3	36-45	46-58
TYPE OF DWELLING, TOTAL	5844	2234	3610	310	704	1010	1	1092	172
CONC. HOUSE	25 9	27 0	25	27 5	17 3	33	23 6	18.2	30.5
FLAT	48 1	49 5	7 8	38 8	31 3	83	87 9	44.5	5
DIFFERENCE	25.9	23.8	2	33.7	81	13 2	30 5	3	1 9
NUMBER OF ROOMS, TOTAL	5 1	2238	3403	508	710	1 1	13	189	1 1
CONC. -2 ROOMS	11 8	12.9	11 8	9 7	11 7	14 5	9 2	8 9	16.1
3-	64.	45.4	3 8	76 2	0.	3 8	73 1	59	6 9
DIFFERENCE	23	22 1	2 9	10 1	27 9	20 0	21	31	2 0
ETC. -2/3-	6 2	9 4	6 7	7	5	4	5	7 8	3
NUMBER OF PERSONS LIVING IN THE DWELLING TOTAL	5873	2250	3623	90	709	1 32	1	1182	1711
CONC.	2 2	2 9	1	1	2	3 3	1 0	1.4	2
3-	85.2	9.	84	9 0	80.8	8 3	3 1	83 2	85
DIFFERENCE	12	11 8	13 9	7	15.	2	14	13 2	12 2
CONC. 1/4-	5	3	6	4		1	1	1	5
[illegible], TOTAL	5885	225	3628	508	704	1 2	8C	09	1725
CONC. YES	22 8	23	21 2	21 1	13	3		3	2
NO	4 5	1.3	8.4	35.8	55	54	36	1	2 1
DIFFERENCE	24 5	25.3	30	0.	31 2	1 8	44	7 8	1
DWELLING-SATISFACTION TOTAL	5844	2245	361	505	3	1	10	895	71
CONC. NOT SATISFIED	1 8	1.9	1 8		1	2 8		1 5	2 5
SATISFIED	84.	5.	8	90.	88	84 2	87	2	84 1
DIFFERENCE	13 3	12.9	1 3	5	1 7	1 0	11 2	1 8	13
[illegible] OF RESIDENCE	5 57	2209	5			1 5	7 3	107	16 1
CONC. NOT SATISFIED	1.6	2 1	1 3		1 3	5		7	2
SATISFIED	8 1	88 5	86 2	93 1		84 1	91	3.3	82 3
DIFFERENCE	11	9.	12	6.9	10.2	10 3	8 3	11 0	15.

TABLE 3. RESIDENCE SITUATION SENSE OF WELL-BEING ETC. MEN CON

	MEN % TOTAL	NZ	OZ	MEN 26-35	36-45	46-58	MEN % 26-35	36-45	46-5
NEIGHBORHOOD, TOTAL	5733	33	320		97	10	7 1	106	1 73
CONC. NOT SATISFIED	1		1 5		3	.8		1	1
SATISFIED	84.	8	84	0	3.		89	83 8	1
DIFFERENCE	13			0	5 5	2 1		15.1	15 8
NEIGHBORS SATISFACTION [illegible] TOTAL	56	2 3	34	3	84	1005	781	104	1641
CONC. NOT SATISFIED	1 8		3			3.		0	2
SATISFIED	84.8	8.5	8	92 7	85.	8	93	8 9	2
DIFFERENCE	9	5	1 2		9.	10.	0	10	11
TRANSPORTATION [illegible] TOTAL	565	1	3475	480	682	1 76	8	1852	1663
CONC. NOT SATISFIED	11	2.	11.4	1	8.			7	13.0
SATISFIED	60	1	8	66.	56.	7	1 2	56 7	7
DIFFERENCE	2	5	29	2	33.	1.4	10 1	33	25.
[illegible], TOTAL	5273	205	3220	3	89	7	7 1	1042	1 17
CONC. NOT SATISFIED	2 8	1.	2	6	9	8			
SATISFIED	83.4	84.	82	89	86 1	1.8	88	84 5	77 8
DIFFERENCE	3	1	2	8	1.5	1 2	10 0	3	2 .2
WORK ENVIRONMENT, TOTAL	5277	2061	321	85	1	8	762	031	1423
CONC. NOT SATISFIED		5.	3	1 7	5.9	7	1 6	.6	.2
SATISFIED	72	4.	1 3	80 2		1 3	78	71	47 8
DIFFERENCE	22	19.	25	1 2	18 7	21	1 7	25.2	2 0
SERVICE ESTABLISHMENTS TOTAL	5	5	341	484	693	97	7 7	1050	1998
CONC. NOT SATISFIED	3		.2	5	3	15	3 9	2.8	10.2
SATISFIED		9.	64 5	74 1	69.2	7.8	71 2	44.2	4.
DIFFERENCE	25	2 3	2	20.	26.	22 5	2	28	23 3
PARKS AND RECREATION AREAS TOTAL	5648	2 89	3479	92	96	1000	45	1042	2427
CONC. NOT SATISFIED	3.	4.8	2 8	1	7	8	8	2 8	.6
SATISFIED	77 2	8.1	7	82 1	7 2	7 7	83.2	7 8	74.1
DIFFERENCE	1 5	18.	20	16.1	20.1	17	1 0	22.8	21 5

T BLE B12 EDUCATION OCCUPATION AND WORK SITUATION WOMEN

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 26 35	WOMEN MZ 36–45	WOMEN MZ 46 58	WOMEN DZ 26 35	WOMEN DZ 36–45	WOMEN DZ 46–58
EDUCATION MANDATORY 9 YEARS OR LESS									
TOTAL	68	713	142	406	874	12 3	1352	1296	1711
CONC. ABOVE	38 0	37 6	33 2	46 2	33 1	34 9	9 1	73 9	35 1
HIGHER EDUCATION	1	44.9	39 C	37 0	47 5	1	2 2	1 4	7
DISCORDANCE	19 0	14 5	2 9	16 2	18 1	11 1	25 7	3.8	18 1
PRESENT GAINFUL EMPLOYMENT									
TOTAL	6466	2 69	3897	378	838	1153	16	123	1649
CONC. FULL TIME	1 3	21 4	17 4	1	17 4	27	12 2	19	22 2
PART TIME	5	1	7 6	17 8	11 5	3 3	19 7	9	2 7
NOT GAINFULLY EMPLOYED	5 5	24 9	24 6	13 3	19 0	39	13 1	1 1	3 4
DISC FULL/PART	14 0	12 9	14 7	1 6	16 1	7 3	24 4	15 7	8 8
FULL/NOT	19 7	18 4	20 7	1 0	19 3	18	16 5	20 2	23.5
PART/NOT	13 5	11 2	15 0	17 0	16 7	4 8	20 2	20 6	7 9
CHANGE OF EMPLOYER DURING THE LAST									
TEN YEARS TOTAL	4844	58	2 34	409	4 9	84	70	4	1294
CONC 4 TIME(S)	2 1	2 3	1 9	2	1 3	4 1		7	0
0–3	8 0	85.4	84 7	96 8	84 8	80	96 5	85 5	77 3
DISCORDANCE	12 9	12 1	13	2 9	13 8	15 0	3 9	13.8	19 7
D SC 3/4	1 6	1 8	1 5		1 9	2 6	1	1 7	1 6

TABLE B12 EDUCATION OCCUPATION AND WORK S TUAT ON WOMEN CONT'D

	WOMEN TOTAL	WOMEN MZ	WOMEN DZ	WOMEN MZ 2 35	WOMEN MZ 3 –45	WOMEN MZ 58	WOMEN DZ 2 35	WOMEN DZ 36–45	WOMEN DZ 4–55
OVER-TIME AT PRESENT									
TOTAL	5	2150	3 14	2	7	10 3	7 7	97	144
CONC YES	1	7	1 1		5	2	1	1	
NO	8	8 8	85 1	4	86 0	7	83 0	84	
D SCORDANCE	13 0	11 8	13 8	13 0	11 5	11	15 8	1 0	12 5
EXTRA JOB NOW OR EARL ER									
TOT L	57	18	3464	590	7 0	17	9 1	114	1 1
CONC. YES	C	0	3 3	1	2 0	3	[illegible]	1 0	7
NO	80 8	93.7	83 8	87	8C 5	7	5 1	1	
DISCORDANCE	15 2	14.3	15 8	11 2	17	13	13 3	1 5	1 7
SH FT–WORK NOW OR EARL ER									
TOT L		2331	3 72	502	7	1C	4 5	1 J9	157
CONC. YES	8	7 9	2 5		2		9	2 0	3 2
NO	83 3	9 5	81 9	84 3	81 8	87	2 5	0.	1
DISCORDANCE	13 8	11 2	15 6	1 3	13	9	15	17 1	1
PIECEWORK NOW OR EARL ER,									
TOTAL	561	547	1 70	11	164	13	8 2	111	1 15
CONC. YES	2	5	0	9	7 7	5	9 3		
NO	79	1 1	7C 5	7 3	7 0	8	7 7	77	87 8
D SCORDANCE	1 2	17	15 5	1 9	1 3		21 0	15	12 5
UNEMPLOYED NOW OR EARL ER									
TOT L	3 05	253	3 01	593	7 3	10	4	104	1 12
CONC. YES	2	4.1	2 7		1 5	5	7		
NO	8 8	85 6	8 2	8 7	85	81	91 3	88 2	
D SCORDANCE	12 4	11 2	13 1	7	1	13	8 3	11 5	17 0

TABLE 41. RESIDENCE & TUITION, SENSE OF WELL-BEING, ETC.

	TOTAL			25-35	36-45	46-5	26-35	36-4	46-5
TYPE OF DWELLING, TOTAL	644	225	3648	318	706	1039	81	1093	1773
PERC. HOUSE	23 9	2 8	25 3	27 5	17 3	3 4	21 6	18 2	30
NOT	8 1	5	7 3	38 3	31 3	5	37 5	44.5	3
INDEPENDENCE	25.	23.5	27.4	23.7	31 4	13 8	38 5	37 8	19 9
NUMBER OF ROOMS, TOTAL	95 1	2238	346	88	710	1 1	18	1099	1 31
PERC. -4 ROOMS	11	12.5	11 3	9 7	11 7	1	5 2	8	16.1
5-	44	5.	3	74.2	60	3 5	73 1	5	41
INDEPENDENCE	23 8	22 1	2	1 1	2 9	20 8	21 9	31	22 0
ETC. 3/5-	6 2	5.	6	3	6.3		5	7	8
NUMBER OF PERSONS LIVING IN THE DWELLING, TOTAL	94 3	2210	3623	608	09	1 32	81	1102	1711
PERC.	2 2	2	3 5	1	.2	1 5	1 0	1	2 3
3-	85 2	5.	84 7	90 8	0.8		85 1	83.2	8
INDEPENDENCE	12	11 2	13 8	8 7	15	2	1	19 2	12 2
ETC. 1/4-		3	6	4		1	1	1	5
RENT, TOTAL	8 5	225	3628	08	706	10 2	8C	109	1728
PERC. YES	22	23	21 2	31 1	13 5	31 3	1 8	11	2 8
NO	49 8	1 3	8	39	55	8	26 8	81	52 1
INDEPENDENCE	2 5	23 3	32	3.	31 2	1 8	44 2	3	18 9
DWELLING-SATISFACTION TOTAL	584	2249	362	909	7 8	1 1	10	1095	171
PERC. NOT SATISFIED	1 8	1 9	1 3		1	2 8		1	2 8
SATISFIED	84.9	95.	84	90.	83.	2	87	82	6 1
INDEPENDENCE	1 3	12	1 .4	8	14.7	1 8	11 2	14 2	13.
NEIGHBOURHOOD OF RESIDENCE	5 5	10	39	49	9	10 5	78	107	1 1
PERC. NOT SATISFIED	1.4	2 1	1		1				2 3
SATISFIED	7 1	88 3	86 2	93 1	8	9 1	91	88.	82 5
INDEPENDENCE	11 3	9.	12	6.	10.2	10 3	8 3	11 8	15

TABLE 41. RESIDENCE & TUITION, SENSE OF WELL-BEING, ETC.

	TOTAL			26-3		46-50	26 35	36-45	46-5
NEIGHBOURHOOD, TOTAL	5723	22 3	3570	9	7	18	7 1	047	1472
PERC. NOT SATISFIED	1	1 8	1 8		1 1			1	1
SATISFIED	84	89.	5	8	1.	5	89	83 8	83 1
INDEPENDENCE	3	12	1	10	15 5	2	9	8	15.8
RE... SATISFACTION ... TOTAL	94 4	2 93	34 1	49	604	1009	81	849	1 1
PERC. NOT SATISFIED	1 5	1 9	1 3					1.8	1
SATISFIED	88.5	8.5	8	92	85.	8	91 6	8 5	8 2
INDEPENDENCE	9		10.2	4.	9.	1	8	10.4	11
TRANSPORTATION ... TOTAL	94 2		34	488	82	1094	7 8	1052	1643
PERC. NOT SATISFIED	1	12.8	11.4	1	10	9	8 7	7 8	19.0
SATISFIED	60	1 6	2 1	64	64	42	2 2	54 7	8 7
INDEPENDENCE	20.	2	29	2	3	21	10 1	35	28.3
... TOTAL	9273	2051	322	9	89	78	7 1	1042	1 17
PERC. NOT SATISFIED	2.3	3.			2 8	8 8	7	8	.4
SATISFIED	82.	94.	2 5	7	86 1	81.0	89	84 5	77 3
INDEPENDENCE	3 8	11.	1		11	1 3	1 8	1	8.3
... ENVIRONMENT, TOTAL	9277	2041	321	84	492	84	62	1031	1423
PERC. NOT SATISFIED	8	5.	4 3	1	5.	7	1	1	.2
SATISFIED	7		71 8	80 2	3 4	71 8	78 7	71 2	8
INDEPENDENCE	22	19.	25 3	1 2	8.	21	1 7	23 2	2 0
SERVICE ESTABLISHMENTS, TOTAL	9580	5	3418	494	693	75	67	1058	1598
PERC. NOT SATISFIED			2	3	3	1 6		5.	10.2
SATISFIED		9.8	64 8	4.1	6 2	8	72 2	4 2	5
INDEPENDENCE	25 1	23.	26	20.4	26.5	22	24	28	25 3
PARKS AND RECREATIONAL AREAS, TOTAL	5668	21 8	3479	92	696	1800	788	1062	1687
PERC. NOT SATISFIED	3	4.8	2	1 8	2 7	8	8	2 0	4.4
SATISFIED	77 2	78.1	7 7	82 1	7 2	7 7	3 2	76.0	74.1
INDEPENDENCE	19 8	18.8	28	16.1	20.1	17	16	23.0	21 5

TABLE B12 EDUCATION OCCUPATION AND WORK SITUATION WOMEN

	WOMEN MZ DZ TOTAL	WOMEN MZ DZ MZ	WOMEN MZ DZ DZ	WOMEN MZ 26 35	WOMEN MZ 36–45	WOMEN MZ 46 50	WOMEN DZ 26–35	WOMEN DZ 36–45	WOMEN DZ 46–55
EDUCATION MANDATORY 9 YEARS OR LESS									
TOTAL	62	713	1 2	406	874	1230	1092	1296	17 1
CONC. ABOVE	38 0	37 6	33 2	46 2	33 1	36 5	9 1	9 4	1 1
HIGHER EDUCATION	1	44.5	37 C	37 0	47 5	51	2 2	1 4	7
DISCORDANCE	19 0	14 5	21 9	16 2	18 1	11 1	25 7	25.8	11 3
PRESENT GAINFUL EMPLOYMENT									
TOTAL	4464	2 69	3897	578	818	1193	46	127	1
CONC. FULL TIME	1 0	21 4	17 4	1	17 4	27	12	19	22 2
PART TIME	9	1	7 6	17 8	11 5	9 3	13 7	4	7 7
NOT GAINFULLY EMPLOYED	5 5	2 9	24 6	13 3	19 0	39	13 1	1 1	3 6
DISC FULL/PART	14 0	12 9	14 7	19 6	16 1	7 3	26 4	15 1	8 6
FULL/NOT	19 7	18 4	20 7	17 0	19 3	18 4	16 5	20 2	23 5
PART/NOT	13	11 2	15 0	17 0	14 7	4 9	20 2	20 6	
CHANGE OF EMPLOYER DURING THE LAST TEN YEARS TOTAL	4894	1 58	2 34	409	679	84	709	49	1274
CONC 4– TIME(S)	2 1	2 3	1 9	2	1 3	4		7	0
0–3	8 0	85.4	8 7	96 8	84 8	80	96 5	5 5	77 3
DISCORDANCE	12 9	12 1	13	2 9	13 8	15 0	3 9	13.8	15 7
DISC 3/4	1 6	1 8	1 5		1 9	2 6	1	1 7	2 C

TABLE B12 EDUCATION OCCUPAT ON AND WORK SITUAT ON WOMEN CONT D

	WOMEN MZ DZ TOTAL	WOMEN MZ DZ MZ	WOMEN MZ DZ DZ	WOMEN MZ 26 35	WOMEN MZ 36–45	WOMEN MZ 46–50	WOMEN DZ 26–35	WOMEN DZ 36–45	WOMEN DZ 46–55
OVER-TIME AT PRESENT TOTAL	5	2150	3 10	2	7	10	7 9	7	14
CONC. YES	1	2	1 1		5	2	1	1	
NO	8 4	85.8	85 1		8 0	86	83 0	6	
D SCORDANCE	13 0	11 4	13 8	13 0	11 5	11	15 8	1 0	12 5
EXTRA JOB NOW OR EARLIER, TOT L	97	18	3464	950	790	1	9 1	11	1 1
CONC. YES	C	0	3 3	1	2 0	9	6	1 0	7
NO	80 8	90 7	83 8	8	82 5	7	5	2	1 9
D SCORDANCE	19 2	14.3	15 8	11 2	17	13	13 9	1	
SHIFT-WORK NOW OR EARL ER TOTAL	9	2331	3 72	502	7 9	1	9 8	1 04	192
CONC. YES	8	3 3	2 5		2		9	2 0	1 2
NO	83 3	9 5	81 9	84 3	83 8	87	82 9	9	2 3
DI CORDANCE	13 6	11 2	19	1 3	13	9	19	8 1	1
P ECEWORK NOW OR EARL ER, TOT L	561	147	3 70	11	764	13	2	117	1 15
CONC. YES	2	5	0		7 7	9	3		
NO	79	1 1	76 5	7 3	78 0	9	3 7	77	87 0
D SCORDANCE	14 2	1	15 9	1 9	1		21 0	15	17 5
UNEMPLOYED NOW OR EARL ER TOTAL	5 75	290	3 01	5 3	7 3	10		104	1 12
CONC. YES	2	3.1	2 7		1	5			
NO	8	8	8 2	8	8	81	1 0	8 2	
DISCORDANCE	12	11 2	13 1	7	3	19	8 3	11 9	1 0

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TAULE 013   PE IDENCE SITUATIO SE E OF WELL-BE G ETC WOMEN

	WOMEN MZ DZ TOTAL	MZ	DZ	WOME MZ 24 35	36-45	46 59	WOME DZ 24 35	3 5	4-59
TYPE OF DWELLI G TOTAL	7 8 31	5 4	67	409	8 6	121	154	97	17 3
CONC HOUSE	2 8	2 9	2	1 9	17 4	5	22 9	17 7	1 3
NOT		51 2	43 1	42 3	46	99	39 2		
DISCORDANCE	6	0	31 2	37 8	35 3	1 7	42 3	30.2	1 .4
NUMBER OF ROOMS TOT L	4 8 03	2649	4115	605	866	121	1092	1287	1771
CONC. 2 ROOM	11 7	12.2	11	5 3	8 3	25	4 0		27 1
3-	61	64	57 8	69 9	66 6	5 7	61 7	1 9	51 6
D SCORDANCE	24 7	73 5	8 8	2 3	2 6	21 0	27 3	30.7	28
DISC. 2/5-	6 6	5 1	7 6	9 4	5 5	4	7 9	8 1	7 1
NUMBER OF PERSONS LIVING IN THE DWELLING TOTAL	4804	7 0	4114	03	8 1	1233	1093	129	1772
CONC. 1	1 7	1 7	1 3	1 0	1 1	2	3	1	
2-	86 7	9 8	8 3	89 1	88 6	88	8 6	87 1	8 5
DISCORDANCE	11 6	9 4	11 0	10.0	10 2	8 7	10 2	11	13
DISC. 1/6-	6	3	4	8	5	3	1 2	3	3
PET . TOTAL	479	890	100	5 8	864	1223	10 2	1276	1775
CONC. YE.	23 3	23 5	23 2	21 1	15 3	30	24 1	16 3	27 5
NO	46 6	7 9	5 1	40 0	50 2	52	34 0	44 8	51 8
DISCORDANCE	30 1	2 4	31 7	39 0	34 5	17 9	41 9	38.7	20 7
DWELLING-SATISFACTION TOTAL	677	2 98	0 6	605	850	1219	1036	127	177
CONC. NOT SATISFIED	1 C	2.9	1 9	7	5	3	9	1 0	3 1
SATISF ED	86 3	87 8	85 3	87 8	89 1	8 0	89 4	86	82 9
DISCORDANCE	11 8	10 2	1 8	11 6	10.5		10 7	12	1 1
MUNICIPALITY OF RESIDENCE TOTAL	45 3	2421	3952	583	840	11	93	1239	1735
CONC. NOT SATISFIED	2 1	2 3	2 0	5	1 0	4 2	1	5	1
SATISFIED	87 1	97 1	87 0	91 8	89 2	83	9 1	8	81
D SCORDANCE	10.8	10 5	11 0	7 7	9 9	12 4	5 8	10 1	14.5

TABLE B13   RESIDENCE S TUAT ON SENSE OF ELL-BEING ETC WOMEN CONT D.

	WOMEN TOT L	MZ	DZ	WOMEN MZ 24-35	3 5	4 59	WOME DZ 2 35	3 -45	4 59
NE GHBORHOOD TOTAL	1	2	969		338	11	16	1231	1
CONC NOT SAT SFIED	1	1 8	1 4		7	5		8	7
SATISF ED	9.	8	6	4	83 9	95 2	1 7	8	83 9
D SCORDANCE	1 3	1 2	13 0	11 0	10.7	11 5	7	3	1
NEIGHBOR SATISFACTION WITH TOT L	6514	7 4	1920	594	92	11	7 8	1208	1 71
CONC. NOT SAT SFIED	1 4	1.9	1 4	2	7	5	1		7
SATISFIED	85 2	5 9	88 4	92 8	91	9	92	3 1	3 3
D SCORDANCE	10	13.3	0 2	0	7 9	15	7 5		1
TRANSPORT TION FAC LIT ES TOTAL	634 3	2542	3818	49	799	11 2	9	1182	1
CONC. NOT SATISF ED	3	2	8 6			1 0	7		12 3
SATISF ED	7	64 7	63 0	69	65 7	63	5	0 2	1
D SCORDANCE	7 3	75 1	28	27 2	23	25 1	2 8	12	2 1
WORK, TOTAL	5 0.	20	3112	67	5.		1	9.	159
CONC. NOT SATISF ED	1	3	2	2	9				3 3
S T SF ED	8 3	9 3	6	3	91	9 1	1	1 0	8
DISCORDANCE	10 8	3	11 8	7	2	11	7	8	7
WOR ENV RO ENT, TOTAL	5 1		3055	44	2		775	3	154
CONC. NOT SATISF ED	2	3	1	1	1 6				1
S T SF ED	8 0	9	3 7	8 3	87 1				7 3
DISCORDANCE	13	1 1	3 3	2	11	1		8 12 9	
SENS OF ESTABL SHMENTS TOT L	3	3	2	9	76	1	2 1	3	1
CONC NOT A SF ED	9	6	3	9 1		1			5
SA SF ED	9	8	43 7	72	7 2	1	2 7	7	8
DISCORDANCE	2 1	27 6	29 1	22 5	33 1	2			
HS AND RECREAT ON AREAS TOT	6	3	1 3			11	96	11	1737
CONC NO S SF ED	1		3 9	1			1 7	1 8	
S T		0	75	3 7	1	79	82 1	8	
D CORDANCE	1 3	7 7	23	1	17 7	17	8 1		23 6

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TABLE B13 RESIDENCE SITUATION, SENSE OF WELL-BEING ETC, WOMEN

	MEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
TYPE OF DWELLING TOTAL	831	25 4	C7	409	8	121	104	8	17 1
CONC, HOUSE	8	2 9	2	1 9	13 6	5	2 8	17 2	1 0
FLAT		51 2	8 1	42 3	44	58	5 3		
DISCORDANCE	6	0	31 2	37 4	35 3	1 7	42 3	18 2	1
NUMBER OF ROOMS TOTAL	4803	6 9	4115	605	366	121	1052	1297	17 1
CONC, 2 ROOMS	11	12 2	11	5 8	4 3	1 1	4 0		2 1
3-	1	6	57 8	69 9	66 6	4 7	61 7	1	31
DISCORDANCE	7	3 5	8 8	2 3	26 6	21 3	27 3	30.7	26
DISC, 2/5-	6 6	5 1	7 6	5 6	5	4 4	7 9	8 1	7 7
NUMBER OF PERSONS LIVING IN THE DWELLING TOTAL	4804	7 0	4114	603	8 1	1213	1053	12 6	1772
CONC, 1	1 7	1 7	1 4	1 0	1 1	2	3	3	
2-	86 7	88 6	85 3	89 2	88 6	88	81 6	67 1	8 5
DISCORDANCE	11 6	9 4	13 0	10 0	10 2	8 7	10 2	11	15
DISC, 1/6-	6	5	6	4	5	1	1 2	3	9
PETS TOTAL	6793	490	100	5 8	864	1225	1042	1276	1779
CONC YES	25 3	23 5	3 2	21 1	15 3	30	24 1	16 9	2 5
NO	44 6	49 9	5 1	40 0	50 2	52 4	34 0	44 8	51 9
DISCORDANCE	30 1	2 4	31 7	39 0	34 5	17 1	41 9	38 7	20 7
DWELLING-SATISFACTION TOTAL	6772	2 96	036	605	850	1219	1036	127	177
CONC NOT SATISFIED	1 9	1 9	1 9	7	4	3	9	1 0	1 1
SATISFIED	86 3	8 8	85 3	8 8	89 1	8 0	89 4	86 7	82 8
DISCORDANCE	11 8	10.	1 8	11 6	10 5		10 7	12	1 1
MUNICIPALITY OF RESIDENCE TOTAL	6573	2621	3952	583	840	11 6	91	1233	1735
CONC NOT SATISFIED	2 1	2 3	2 0	5	1 0	4 2	1	4	1
SATISFIED	8 1	87 1	87 0	91 8	85 2	83	94 1	89	81
DISCORDANCE	10.8	10.5	11 0	7 7	9 9	12 4	5 8	10 1	14.1

TABLE B13 RESIDENCE SITUATION, SENSE OF WELL-BEING ETC, WOMEN, CONT D.

	MEN TOTAL	MZ	DZ	WOMEN MZ 26-35	36-45	46-58	WOMEN DZ 26-35	36-45	46-58
HOME CHILDHOOD TOTAL	6 1	6.2	969		338	11		1231	1
CONC, NOT SATISFIED	1	1 8	1 4		7	3		6	7
SATISFIED	96	0	5 6	4 8	83 5	8 2	1 7	8	32
DISCORDANCE	1 3	1 2	13 0	11 0	1 7	11 5	7 9	1	1
NEIGHBOURS SATISFACTION WITH TOTAL	651	2	1920	59	926	11	9 9	1239	1 21
CONC, NOT SATISFIED	1 6	1 9	1 4	2	7	3	1		7
SATISFIED	88 2	9 9	88 4	92 8	91 4	8	92	3 1	5 3
DISCORDANCE	10	13.3	3 2	0	7 9	13	7 9		12
TRANSPORTATION FACILITIES								1182	1 9
TOTAL	43 0	2542	3018	549	799	1172	7		12 1
CONC, NOT SATISFIED	3	2	8			1 9	6 4	0.2	1
SATISFIED	7	64 7	63 0	69	63 7	63	26 8	32	1 1
DISCORDANCE	7 1	7 1	28	27 2	28	21 1			
WORK, TOTAL	5.9	209	3112	467	94		31		13 3
CONC, NOT SATISFIED	1	3	1	2	9				3 2
SATISFIED	6 3	9 3	9 6	9 3	91	9 1	91 9	1 0	8
DISCORDANCE	10 8	3	11 8		7 2	11	7	6	8
WORK ENVIRONMENT TOTAL	5 1	44	3085	8	2		75	5	11 9
CONC, NOT SATISFIED	2	3	1	1	1				1
SATISFIED	8 0	94.4	83 7	8 3	87 1	8	8 9		7 1
DISCORDANCE	13	1 1	1 3	2	11	1	8	12 5	5
SERVICE ESTABLISHMENTS TOTAL	634	1	2			1	2 1	3	1
CONC NOT SATISFIED	9		6 3	1		1		0	
SATISFIED	9	8	63 7	2	7 3	1	3		2
DISCORDANCE	2 1	22	25 1	72 5	23 1	22	22 7	27	8
AREAS FOR RECREATIONAL ... TOTAL	3	3	3 3		8	13	9	11	17 7
CONC NOT SATISFIED	1		9	1	1		1 2	1	
SATISFIED	7	0	5		6	74 4	87 1		
DISCORDANCE	5	7 7	23 9	1 5	17 7	17	0	1	27 1

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QUESTIONS ABOUT YOURSELF AND YOUR TWIN PARTNER

<u>QUESTIONS ABOUT MARITAL STATUS, NUMBER OF SIBLINGS AND NUMBER OF CHILDREN</u>

1 Are you a twin ?

○ no ⟶ if no go directly to question 5
○ yes

2 Were you and your twin partner during childhood as alike as "two peas in a pod" or "did you bear no more resemblance to each other than siblings in general" ?

○ like two peas in a pod
○ "no more resemblance than siblings in general"
○ do not know

3 How long did you live together with your twin partner?

○ I am still living with my twin partner
○ I lived together with my twin partner until the age of ______

4 How often do you communicate with your twin partner?

○ daily or almost daily
○ one time or so per week
○ one time or so per month
○ less often
○ never

5 When were you born?

year	month	day

6 Are you

○ single ?
○ married/cohabiting?
○ divorced?
○ widow/widower?

7 Do you have any siblings (in addition to your twin partner)?

○ no
○ yes ⟶ How many?
______ siblings

8 Do you have any children?

○ no
○ yes ⟶ How many?
______ children

Do you have or have you had any ch [illegible] tw
no
yes

<u>QUESTIONS ON YOUR HEALTH STATUS</u>

9 Have you ever had any pain or discomfort in your chest?

○ no ⟶ if no go directly to question 11
○ yes

10. Have you felt pain or discomfort more often than just on exceptional occasions?

○ no ⟶ if no go to question 11
○ yes

a When do you feel this pain or discomfort?

○ Virtually at any time whatsoever
○ When you are emotionally upset or excited
○ When you walk fast or walk uphill
○ When you walk at a normal speed on level ground
○ Under other circumstances

b What do you do if you get this pain or discomfort while you are walking?

○ stop or walk more slowly
○ take medicine and continue walking at the same speed
○ continue at the same speed without taking medicine

If you stop walking regardless of whether or not you take medicine how is happens the pain or discomfort [illegible] ?

○ The pains usually pass within 10 minutes
○ The pains usually continue for more than 10 [illegible]

c Where are the pains or the discomfort [illegible]

○ in the middle of the chest
○ in the left side of the chest
○ in the left arm
○ in some other place

11 Do you have a cough regularly or for extended periods of time?

○ no ⟶ if no go directly to question 12
○ yes

For how many months in a row per year do you have [illegible]

○ fewer than three months in a row per year
○ more than three months in a row per year

b For how many months in a row per year do you cough up phlegm from your chest?

○ fewer than three months in a row per year
○ more than three months in a row per year

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29 Which BRAND OF CIGARETTES do you normally smoke now or did you normally smoke just before you stopped?

________ brand of cigarettes

30. In your entire life have you smoked more than 50-75 CIGARS/CIGAR-CIGARETTES or 3-8 packages of PIPE TOBACCO or used more than 3-6 cans of SNUFF/CHEWING TOBACCO?

0 no → if no go directly to question 35
0 yes → if yes answer the following four questions for each tobacco type you use or used

		Cigars/ cigar-cigarettes	pipe tobacco	snuff/ chewing tobacco
31	Put an X in box indicating tobacco type (s) you used or used	0	0	0
32.	State how old you were when you started using the type(s) which you used or use	___ years old	___ years old	___ years old
33	If you stopped using one or several of the types you have checked state how old you were when you stopped	___ years old	___ years old	___ years old
34	State how much you use now or how much you used just before stopping with regard to each type(s)	number/day	packages/week	cans/week

DRUG CONSUMPTION

We are interested in knowing whether you use or have used the following types of medicine and if so how often? Put an X to indicate one of the following three alternatives in each line

		almost never	now and then	regularly for shorter or longer periods
35	Bitter tinctures or supplements like iron preparations and [illegible]	0	0	0
36	Press [illegible]-free [illegible]			0

39 Do you use now or have you earlier used any other pharmaceutical regularly?

0 no
0 yes → What pharmaceutical? ________

40. Do you now use contraceptive pills? (to be answered by women only)

0 yes → How long have you used contraceptive pill ? ____ years
0 no → Have you earlier used contraceptive pills?
0 no
0 yes → How long did you use contraceptive [illegible] ____ years

DRINKING HABITS

41 Do you drink	No	Yes	
MEDIUM-STRONG OR STRONG BEER	0	0	____ bottles/month
WINE	0	0	____ bottles/month
LIQUOR	0	0	____ bottles/month

42 Is your consumption of wine and liquor for the most part concentrated to holidays and weekends, or is it spread throughout the entire week or do you drink only on individual occasions?

0 Mostly during weekends and holidays
0 For the most part spread throughout the week
0 Only on individual occasions → Go to question 44
0 Do not drink at all → Go to question 44

43 Does it happen that at least once or so a month and on the same occasion you drink more than 5 bottles of beer, or more than one bottle of wine or more than half a bottle of liquor?

0 no
0 yes

44 During any earlier period have you habitually drunk more than you [illegible]

0 no → If no go directly to question 46
0 yes

45 At the time you drank most how much did you drink of

MEDIUM-STRONG OR STRONG BEER ____ bottles/[illegible]
WINE ____ bottles/month
LIQUOR ____ bottles/[illegible]

12 Do you usually feel shortness of breath when you walk uphill on small hill or when you walk rapidly on flat terrain?

0 no 0 yes

13 Have you ever had a severe pain across the front of your chest lasting 30 minutes or more?

0 no 0 yes

14 During the last years have you had feelings of discomfort burning or achiness in the diaphragm?

0 no 0 yes

15 During the last years have you had so much pain in the back shoulders or neck that you found it difficult to work?

0 no
0 yes in the back
0 yes in the shoulders
0 yes in the neck

16 During the last years have you had recurring headaches which have been so severe that you have found it difficult to work?

0 no ⟶ if no go directly to question 17
0 yes

a Is the headache usually accompanied by visual disturbances or vomiting?

0 no 0 yes

17 Is your hearing impaired?

0 no 0 yes

18 As a child or adult have you had symptoms of asthma false croup hay-fever or eczema?

0 no ⟶ if no go directly to question 19
0 yes

a Do you know whether any or several of the symptoms were brought about by some agent in the environment?

0 no
0 yes ⟶ specify __________

19 How many times per year do you usually catch a heavy cold?

__________ times per year

20 Have you ever had any long term or serious illness?

0 no
0 yes ⟶ what was the illness? __________

21 Have you ever been registered for sick leave for more than 3 months in a row?

0 no
0 yes ⟶ What was the reason? __________

PHYSICAL ACTIVITY, HEIGHT AND WEIGHT

22 Different types of work require different degrees of physical exercise. Try to classify your daily work according to the following alternatives

0 mainly sedentary work
0 work which requires quite a bit of standing and walking but which does not demand other physical activity
0 work which requires standing and walking but also requires lifting and carrying
0 heavy manual labor

23 Here are 7 alternatives to describe the exercise you get during your leisure time. Which one applies best to you when considering the exercise you get during the year as a whole?

0 virtually no exercise
0 exercise very little
0 exercise rather little
0 do not exercise especially much
0 exercise rather much
0 exercise much
0 exercise quite a lot

24 How tall are you?

______ feet ______ inches

25 How much do you weigh?

______ pounds

SMOKING HABITS

26 In your entire life have you smoked more than 5-10 packs of CIGARETTES?

0 no ⟶ If no go directly to question 30
0 yes

27 Have you ever smoked CIGARETTES regularly i e daily or almost daily?

0 no ⟶ If no go directly to question 30
0 yes ⟶ If yes how old were you when you started smoking cigarettes regularly? ______ years old

28 Do you still smoke CIGARETTES regularly?

0 yes ⟶ how many cigarettes with or without filter do you presently smoke per day?

______ cigarettes with filter/day
______ cigarettes without filte /day

0 no ⟶ how old were you when you stopped?

______ years old

How many cigarettes with or without filte did you smoke per day just before you stopped?

______ cigarettes with filter/day

______ cigarettes without filter/day

29 Which BRAND OF CIGARETTES do you normally smoke now or did you normally smoke just before you stopped?

Brand of cigarettes

30 In your entire life have you smoked more than 50-75 CIGARS/CIGAR-CIGARETTES or 3-5 packages of PIPE TOBACCO or used more than 3-5 cans of SNUFF/CHEWING TOBACCO?

0 no ——> if no go directly to question 35
0 yes ——> if yes answer the following four questions for each tobacco type you use or used

	Cigars/ cigar-cigarettes	pipe tobacco	snuff/ chewing tobacco
31 Put an X in box indicating tobacco type (s) you used or used	0	0	0
32 State how old you were when you started using the type(s) which you used or use	____ years old	____ years old	____ years old
33 If you stopped using one or several of the types you have checked state how old you were when you stopped	____ years old	____ years old	____ years old
34 State how much you use now or how much you used just before stopping with regard to each type(s)	number/day	packages/week	cans/week

DRUG CONSUMPTION

We are interested in knowing whether you use or have used the following types of medicine and if so how often? Put an X to indicate one of the following three alternatives in each line

	almost never	now and then	regularly for shorter or longer periods
35 Restoratives or supplements like iron preparations and vitamins	0	0	0
36 Prescription-free pain-killers	0	0	0
37 Sleeping pills			
38 [illegible]			

39 Do you use now or have you earlier used any other pharmaceutical regularly?

0 no
0 yes ——> What pharmaceutical?

40 Do you now use contraceptive pill ? (to be answered by women only)

0 yes ——> How long have you used contraceptive pills?
_______ years

0 no ——> Have you earlier used contraceptive pills?
0 no
0 yes ——> How long did you use contraceptive pills?
_______ years

DRINKING HABITS

41 Do you drink

	No	Yes	
MEDIUM-STRONG OR STRONG BEER	0	0	_____ bottles/month
WINE	0	0	_____ bottles/month
LIQUOR	0	0	_____ bottles/month

42 Is your consumption of wine and liquor for the most part concentrated to holidays and weekends, or is it spread throughout the entire week or do you drink only on individual occasions?

0 Mostly during weekends and holidays
0 For the most part spread throughout the week
0 Only on individual occasions ——> Go to question 43
0 Do not drink at all ——> Go to question 44

43 Does it happen that at least once or so a month and on the same occasion you drink more than 8 bottles of beer, or more than one bottle of wine or more than half a bottle of liquor?

0 no
0 yes

44 During any earlier period have you habitually drunk more than you do now?

0 no ——> if no go directly to question 46
0 yes

45 At the time you drank most how much did you drink of

MEDIUM-STRONG OR STRONG BEER — bottles/month
WINE bottles/month
LIQUOR bottles/month

FOOD HABITS

65 How often do you eat or drink:

	Less than one time/month	One time or so/month	Seve al times/ month or one time or so/week	Several times/week	Almost daily
Grilled or deep-fried food	0	0	0	0	0
Pan-fried or roasted food	0	0	0	0	0
Pure meat	0	0	0	0	0
Sausage or hot dogs	0	0	0	0	0
Liver kidney blood or other organ food	0	0	0	0	0
Fish	0	0	0	0	0
Shelled seafood	0	0	0	0	0
Rice and rice dishes	0	0	0	0	0
Flour-based foods (porridge dry cereals pancakes etc)	0	0	0	0	0
Egg and egg dishes	0	0	0	0	0
Vegetables and root foods	0	0	0	0	0
Fruit	0	0	0	0	0
Milk sour milk yoghurt or cheese	0	0	0	0	0

66 How many warm meals do you eat per day? _______

67 How many sandwiches do you usually eat per day? _______

68 How many cups of coffee do you usually drink per day? __

69 How many potatoes do you usually eat per day? _______

QUESTIONS PERTAINING TO YOUR CHARACTER, FEELINGS AND ACTIONS

Decide whether yes or "no best correspond to your character feelings and actions Do not spend too much on each question We are trying to determine your immediate reactions

		yes	no
46	Do you like to have a lot of things going on around you?	0	0
47	Are you often uneasy and feel that there is some thing you want without knowing what it is?	0	0
48	Do you almost always have an answer when spoken to?	0	0
49	Are you sometimes happy or sometimes sad without any special reason?	0	0
50	Do you prefer to keep to the background when you are in company with other people?	0	0
51	Do you regard yourself as happy and carefree?	0	0
52	Do you often reach decisions too late?	0	0
53	Do you often feel tired and listless without any special reason?	0	0
54	Do you have a lively manner?	0	0
5	Ca you qui kly desc be your thoughts in words	0	
6	Ar you often lost your own thoughts?	0	0
57	Do you have anything against selling things or asking people for money for some charitable cause?	0	0
58	Are you e t emely sensitive in any respect?	0	0
59	Are you ever too estless to sit st ll?	0	
60	you have d ff c lt es i falling asleep	0	
1	Do you keep th ngs t yourself e cept th good f iends?	0	
62	Do you have any nervous problems?	0	
63	Do you l k to ack jokes nd tell funny tor s to ou f iends?		
	o you sually wor y long t me fte e t es ng incident?	0	

72. The following is a list of environmental agents. In each case indicate whether you are annoyed by the agent at your place of work

	Do not notice it	Notice it but I am not annoyed	Somewhat annoyed	Very annoyed
Noise	0	0	0	0
Vibrations	0	0	0	0
Dust, soot, smoke or gases	0	0	0	0
Odor	0	0	0	0
Poor or glaring lightning	0	0	0	0
Humidity	0	0	0	●
Intense temperature changes	0	0	0	0
Too hot	0	0	0	0
Too cold	0	0	0	0
Unsuitable work postures	0	0	0	0
Other agents. Specify ________	0	0	0	0

73. If you have stated that you are somewhat annoyed or very annoyed by any of the above environmental agents complete the following statement:

I think that ____________ is the most annoying environmental agent at my place of work and it is

0 unbearable
● very annoying
0 rather annoying
0 not especially annoying

and I am annoyed by it

0 incessantly
0 several times/day
0 almost daily
0 less often

WE ARE EXPOSED TO DIFFERENT ENVIRONMENTAL AGENTS WHICH DO NOT NECESSARILY MEAN A RISK FOR ILLNESS BUT WHICH BY MANY PEOPLE CAN BE CONSIDERED ANNOYING.

70. The following is a list of environmental agents. In each case indicate whether you are annoyed by the agent at your place of residence

	Do not notice it	Notice it but I am not annoyed	Somewhat annoyed	Very annoyed
Traffic noise	0	0	0	0
Aircraft noise	0	0	●	0
Industrial noise	0	●	0	0
Noise from neighbours	●	●	●	0
Odor	0	0	0	0
Automobile exhaust/gases	●	0	●	0
Air pollution	●	0	0	●
Dust soot	●	●	●	0
Water pollution	●	0	0	0
Other agent( ) specify ________	0	●	●	●

71 If you have stated that you are somewhat annoyed or very annoyed by any of the above environmental agents complete the following statement:

I think that ____________ is the most annoying environmental agent at my place of residence and it is:

0 unbearable
0 very annoying
0 rather annoying
● not especially annoying

and I am annoyed by it

● incessantly
0 several times/day
0 almost daily
0 less often

FOOD HABITS

65 How often do you eat or drink	Less than one time/month	One time or so/month	Several times/ month or one time or so/week	Several times/week	Almost daily
Grilled or deep-fried food	0	C	0	0	0
Pan-fried or roasted food	0	0	0	0	0
Pure meat	0	0	0	0	0
Sausage or hot dogs	0	0	0	0	0
Liver kidney blood or other organ food	0	0	0	0	0
Fish	0	0	0	0	0
Shelled seafood	0	0	0	0	0
Rice and rice dishes	0	0	0	0	0
Flour-based foods (porridge dry cereals pancakes etc)	0	0	0	0	0
Egg and egg dishes	0	0	0	0	0
Vegetables and root foods	0	0	0	0	0
Fruit	0	0	0	0	0
Milk sour milk yoghurt or cheese	0	0	0	0	0

66 How many warm meals do you eat per day? _______

67 How many sandwiches do you usually eat per day? ______

68 How many cups of coffee do you usually drink per day? __

69 How many potatoes do you usually eat per day? ______

QUESTIONS PERTAINING TO YOUR CHARACTER, FEELINGS AND ACTIONS

Decide whether yes or no best correspond to your character feelings and actions Do not spend too much on each question We are trying to determine your immediate reactions

		yes	no
46	Do you like to have a lot of things going on around you?	0	0
47	Are you often uneasy and feel that there is something you want without knowing what it is?	0	0
48	Do you almost always have an answer when spoken to?	0	0
49	Are you sometimes happy or sometimes sad without any special reason?	0	0
50	Do you prefer to keep to the background when you are in company with other people?	0	0
51	Do you regard yourself as happy and carefree?	0	0
52	Do you often reach decisions too late?	0	0
53	Do you often feel tired and listless without any special reason?	0	0
54	Do you have a lively manner?	0	0
55	Can you quickly desc ibe your thoughts in words?	0	
[illegible]	A[illegible] you often l st [illegible] your own thoughts?	[illegible]	[illegible]
57	Do you have anything against selling things or asking people for money for some cha itable cause?	0	0
58	Are you e t emely sensitive in any respect?	0	0
59	Are you ever too estless to sit still?	0	
60	[illegible] you have d fficult es i falling leep	[illegible]	
61	Do you keep things to yourself e cept w th good f iends?	0	
62	[illegible] you have any nervous problems?	[illegible]	
63	Do you l k to [illegible] ll jokes and tell funny tor s to ur f iends?		
[illegible]	Do you [illegible] ually wor y long time aft [illegible] a e tress g [illegible]	0	

72. The following is a list of environmental agents. In each case indicate whether you are annoyed by the agent at your place of work

	Do not notice it	Notice it but I am not annoyed	Somewhat annoyed	Very annoyed
Noise	0	0	0	0
Vibrations	0	0	0	0
Dust, soot, smoke or gases	0	0	0	0
Odor	0	0	0	0
Poor or glaring lightning	0	0	0	0
Humidity	0	0	0	0
Intense temperature changes	0	0	0	0
Too hot	0	0	0	0
Too cold	0	0	0	0
Unsuitable work postures	0	0	0	0
Other agents Specify ________	0	0	0	0

73. If you have stated that you are somewhat annoyed or very annoyed by any of the above environmental agents, complete the following statement

I think that ____________ is the most annoying environmental agent at my place of work and

it is

- 0 unbearable
- 0 very annoying
- 0 rather annoying
- 0 not especi lly annoying

and I am annoyed by it

- 0 incessantly
- 0 several times/day
- 0 almost daily
- 0 les often

WE ARE EXPOSED TO DIFFERENT ENVIRONMENTAL AGENTS WHICH DO NOT NECESSARILY MEAN A RISK FOR ILLNESS, BUT WHICH BY MANY PEOPLE CAN BE CONSIDERED ANNOYING.

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	Do not notice it	Notice it but I am not annoyed	Somewhat annoyed	Very annoyed
Traffic noise	0	0	0	0
Aircraft noise	0	0	0	0
Industrial noise	0	0	0	0
Noise from neighbours	0	0	0	0
Odor	0	0	0	0
Automobile exhaust/gases	0	0	0	0
Air pollution	0	0	0	0
Dust, soot	0	0	0	0
Water pollution	0	0	0	0
Other agent( ) specify ________	0	0	0	0

75. If you have stated that you are somewhat annoyed or very annoyed by any of the above environmental agents complete the f llowing statement

I think that ____________ i the most annoying environmental agent at my place of residence and it is:

- 0 unbearable
- 0 very annoying
- 0 rather annoying
- 0 not especially annoying

and I am annoyed by it:

- 0 incessantly
- 0 several times/day
- 0 lmost daily
- 0 less often

EDUCATION AND OCCUPATION

74 Have you complete a course of education at any schools beyond elementary school?

0 no
0 yes ——→ What levels of schooling ____________

____

____

75 What type of occupation/employment do you have? (Describe more precisely the type of tasks you carry out for example a welder in the shipping industry a clerk at a grocery store an administrative engineer at the telephone company and so forth)

____

____

76 If you have had some type of work other than the above named during at least 5 years state this occupation (s) below

0 Have not had another type of work
0 yes ——→ Specify ____________

77 Are you gainfully employed at the present time?

0 yes full time
0 yes part time
0 no ——→ Go directly to question 81

78 Where are you employed?

____

____

79 How long does it usually take you to get from your home to your place of work?

0 work at home ——→ go directly to question 81
0 it takes about ______ minutes

80 How do you usually get to work?

0 walk
0 bicycle
0 motorbike motorscooter motorcycle
0 car
0 public transportation

81 How many times have you changed employer during the last 10 years?

_____ times

82 Do you often work over-time?

0 no
0 yes ——→ How much? ________ hours/week

83 Have you now or have you earlier had an extra job?

0 no
0 yes ——→ How much extra work? ________ hours/week

84 Have you now or have you ea lier had shift-work?

0 no
0 yes ——→ During how long a time? ______ years

85 Do you now work or have you earlie worked under a piecework system?

0 no
0 yes ——→ How long? ______ years?

86 Are you now or have you earlier been unemployed?

0 no
0 yes ——→ How long? ______ years

SOME GENERAL QUESTIONS

87 Which different cities have you lived in?

Name of city	County	Pe iod of time	
______	______	19	19
______	______	19__	19__
______	______	19__	19__
______	______	19	19__
______	______	19	19__
______	______	19	19__

88 Do you live in

0 apartment
0 cooperative apartment
0 terrace house (semi-detached) or row-styled housing
0 house
0 other Specify ____________

89 How many rooms (apart from the kitchen) are there in your present dwelling?

______ rooms

90 How many persons live in the dwelling?

_____ persons

91 Do you have any pets?

0 no
0 yes ——→ Specify _____
If dog what breed? _____

92 Are you satisfied with your

	Very satisfied	Rather satisfied	Not especially satisfied	Dis satisfied
Dwelling	0	0	0	0
Municipality	0	0	0	0
Neighbourhood	0	0	0	0
Neighbours	0	0	0	0
Transportation facilities	0	0	0	0
Work	0	0	0	0
Work environment	0	0	0	0
Servic establishments	0	0	0	0
Parks and recreational reas	0	0	0	0

93 Do you experience your daily exi tence as being very stress filled"?

0 no
0 yes ——→ If yes can you name the cause?
0 no
0 yes ——→ Specify _____

EDUCATION AND OCCUPATION

74 Have you compl te a course of education at any schools beyond elementary school?

0 no
0 yes ——→ What levels of schooling ______________________

____

____

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Name of city	County	Period of time	
______	______	19	19
______	______	19__	19__
______	______	19__	19__
______	______	19	19__
______	______	19	19__
______	______	19	19__

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Acta Medica Scandinavica

Supplementum 699

Feasibility of physical training after myocardial infarction and its effect on return to work, morbidity and mortality

By Ilkka Palatsi

Distributed by The Almqvist & Wiksell Periodical Company Stockholm Sweden

From the Department of Medicine, University of Oulu, Finland
(Head: Professor W. J. Kaipainen, M.D.)

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who died of myocardial infarction
during this investigation

Printed in Finland
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Contents

Introduction

There is a note by Heberden that one of his patients was "nearly cured" after 6-month period of sawing wood for half an hour a day. This is the first published observation of a beneficial effect of physical activity in coronary artery disease and is as old as the clinical description of the disease itself. (Heberden 1772)

In the 1950's, patients with myocardial infarction were kept in bed for 4—6 weeks. Levine (1952) with his armchair method, was one of the first opponents of a prolonged confinement to bed. Increasingly short periods of rest in bed were not found to impair the early prognosis, or to increase the number of complications (Brammer 1966). Harper et al (1971) and Takkunen et al (1971) have suggested that confinement to bed for 3—7 days and hospitalization for 12—16 days, depending on the severity of the infarction, would be sufficient. Together with the reduction in bed rest, physical rehabilitation following hospital therapy has been increased and the denial of physical strain for the rest of one life which used to be the ordinary practice, has been replaced by physical activation as early as possible. Kramer and Bruce (1969) and Benestad (1972) noted that the physical working capacity of infarction patients at the beginning of the convalescent period is appr. 30 % lower than the corresponding capacity of healthy subjects of the same age. Hellerstein (1968), Redwood et al (1972) and Kentala (1972), who used a regular supervised rehabilitation of several months duration were able to bring the physical working capacity of infarction patients almost to the level of healthy subjects of the same age. Frick and Katila (1968) and Clausen and Trap-Jensen (1970) have demonstrated the favourable hemodynamic effects of training. Hellerstein and Hornsten (1966) and Rechnitzer et al (1967) have described its beneficial psychic effects.

According to Saltin et al (1968), mere bed rest as such clearly reduces the physical working capacity. Quite naturally the physical working capacity also improves spontaneously after an infarction without any rehabilitation. Kentala (1972) and Sanne (1973) have maintained that working capacity improves more rapidly and to a greater extent through active rehabilitation than without it.

Although there are numerous studies on rehabilitation following myocardial infarction, there is little information so far on the prognostic significance of rehabilitation. The reason for this is that the patient series in most works are selected series, or that the control material is either lacking entirely or is not comparable with the research series. Hellerstein (1968) and Gottheiner (1968) for example, have published works of this kind, which show that rehabilitation reduces morbidity and mortality. Well controlled studies were published by Kentala (1972) and Sanne (1973). Kentala found rehabilitation to have no effect on mortality or morbidity. Sanne noted a difference in mortality in favour of the rehabilitated subjects, but no difference in morbidity. The follow-up period was relatively short in both of these works.

Resumption of work after myocardial infarction is not only a problem of physical rehabilitation, but also a psychic and social problem, which is probably not always solved even by successful physical rehabilitation. If the social conditions are good enough, not as many of those with a low educational background return to work as could be expected on the basis of the results of rehabilitation. Kellerman and Kariv (1968) and Kellerman (1975) in their uncontrolled studies have obtained percentages as high as 85 % for rehabilitated infarction patients returning to their previous employment. Kentala, however found no difference in return to work between the trained subjects and controls.

It is therefore apparent that a patient with myocardial infarction needs physical rehabilitation to be able to return effectively into normal life. Up until now the rehabilitation programs have generally been carried out either in the rehabilitation departments of hospitals or in physiotherapeutic institutes, and patients have attended training sessions several times a week or have lived in the rehabilitation institute. There is only limited knowledge of spontaneous training at home. Uusitalo et al (1972) compared the effects of rehabilitation in an institute and at home in a fairly small patient series. The physical working capacity improved in both patient groups, but more so in the group trained in a physiotherapeutic institute. Return to

Introduction

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Review of the literature

Early mobilization and discharge from hospital

The rehabilitation of a patient with myocardial infarction begins as a bedside therapy at the acute stage, and early mobilization and discharge from hospital are part of the early rehabilitation program.

Bed rest has always been deemed necessary in the treatment of myocardial infarction. The patients ere previously kept in bed for 4—6 weeks, and in hospital for as long as 8 weeks. The traditional notion was that physical exertion increases the risk of cardiac rupture or aneurysm and aggravates hypoxemia, in which case the probability of suffering a reinfarction or developing arrhythmias increases. Restieaux et al (1967) have shown that the main threat to patients, without shock or manifestion of heart failure, is the risk of arrhythmias on the first few days following the infarction. Prolonged confinement to bed, however, involves some disadvantages. According to Nicolaides et al (1971) a long confinement to bed entails the risk of thromboembolic complications, according to Fareeduddin et al (1969) it impairs orthostatic tolerance, and according to Deitrick et al (1948) and Saltin et al (1968), it diminishes the physical exercise tolerance. As early as 1935 Hay described the adverse effects of prolonged bed rest. Groden et al (1970) noted that confinement to bed of shorter duration is beneficial for the psychic well-being of the patient and prevents the development of cardiac neuroses. Prolonged bed rest has therefore become less popular among clinicians, and the current practice is to mobilize the infarction patient as soon as his clinical condition permits it, if no contraindications exist.

Levine ith his armchair method was probably the first opponent of prolonged confinement to bed (Levine and Lown 1952). H made his patients sit in an armchair as soon as possible after the infarction, even on the first post-infarction day and at the end of the first eek of therapy the patients already spent most of their day sitting in the chair. At the end of the third therapeutic eek they ere allowed to walk, and the discharge took place t the end of the fourth eek. Brummer et al (1965, 1966) were the first in Finland to describe series th short therapy. Lown et al (1969) have proposed that hospitalization for 10—12 days would be sufficient. Takkunen et al (1970) described a patient series where bed rest lasted for 3 or 7 days and hospitalization for 12 or 16 days, depending on whether the infarction was intramural or transmural. The control group consisted of patients previously treated in the same hospital, who were kept in bed for 7 or 14 days and in hospital for 21 or 28 days, depending on the severity of the infarction. Seven to 30 days after the infarction there was no difference in mortality between the patients with short therapy and the control group. Harpur et al (1971) discharged their patients on the 15th day of therapy. Eight months after the infarction, there was no difference in the prognosis between them and the control group.

Several different mobilization programs have been suggested (Levine and Lown 195., Newman et al 1952, Caint et al 1961, Torkelson et al 1964, Tobis and Zohman 1968). The program proposed by the WHO working group (1968) is more or less similar to Torkelson's program, which, in turn, is based on that described by Newman. According to this proposal, rehabilitation in hospital can be initiated as soon as the symptoms of shock have disappeared, and there are no symptoms of manifest heart failure and no signs of malignant arrhythmia, when the pains are over and the body temperature is below 39°C.

During the first two days the patient performs breathing exercises in a supine position and maintains venous circulation with light movements of the limbs. On the third day the patient is allowed to sit, but the actual training program involving large groups of muscles is accomplished in a lying position. The patient is permitted to stand at the beginning of the second week, and in the middle of that week breathing and chalistenic exercises are carried out in standing position. The chalistenic program starts from 5 minutes, but is gradually prolonged to 15—30 minutes. On the third week the patient is allowed to alk in the corridor and at the end of the week he can walk up and down stairs. If the heart rate increases by 30 or declines by 10 beats/min during the exercise, if arrhythmias or conduction disturbances develop, chest pain or dyspnea appear the pace of the

work was equally good in the two groups and there were no differences in the prognosis over a 2 year period. There are however no comprehensivly controlled studies on the practicability and the effects of supervised spontaneous training at home For this reason the University Central Hospital of Oulu initiated a rehabilitation project where the rehabilitation of patients with myocardial infarction was largely carried out through spontaneous programmed training at home The intention was to find out whether training of this kind is possible in practice and whether spontaneous "self training" brings about an improvement in the physical condition.

Purpose of the present study

The present work, where men and women aged under 65 who had suffered a myocardial infarction were rehabilitated, sought answers to the following questions.

1 Is rehabilitation based on spontaneous programmed training at home possible in practice?
2. a Does the training group if compared with a control group display a statistically verifiable improvement in symptoms, clinical findings ECG and roentgenological findings, and metabolic risk factors?
b Does the physical condition of the patients improve
c Has rehabilitation any effect on recidivous morbidity and coronary mortality following myocardial infarction?
d. What is the effect of rehabilitation on return to work?

Review of the literature

Early mobilization and discharge from hospital

The rehabilitation of a patient with myocardial infarction begins as a bedside therapy at the acute stage, and early mobilization and discharge from hospital are part of the early rehabilitation program

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A. Central hemodynamics

a. Healthy subjects

1. Heart rate

In 1931 Christensen was the first to note that endurance training brings down the heart rate of healthy subjects at work. Karvonen et al (1957) point out that in order to reduce the working heart rate, the training heart rate must be greater that the resting heart rate + 60 % of the difference between the maximum rate and the resting rate. For example, maximum heart rate 190 resting heart rate 70 training heart rate ≥ 70 + 60/100 × 120 ≥ 142. Hoffman and Venrath (1963), however noted that even an exercise where the heart rate rises up to 115—125/min results in decline of both the working heart rate and the resting heart rate. Åstrand (1960) and Andersen et al (1971) have demonstrated that the maximum heart rate declines along with increasing age. The maximum pulse rate of a 20-year-old is appr 200/min and that of a 60-year-old only appr 160/min. Thus, older people reach their maximal aerobic capacity at a lower heart rate, and the training effect is similarly achieved at a relatively lower heart rate. According to Roskamm (1967), a 30-minute training session 5 times a week for one month, at constant 70 % load, brings about a maximal decline in the working heart rate measured by the same load. Roskamm maintains that the best increase in functional capacity is reached if the same amount of ork is done in 30 minutes, but the load is alternately increased and decreased by 50 % at intervals of 2 1 2 minutes. Saltin (1970) observed that a 30-minute training session 2—3 times a week brings down the orking heart rate nearly as much as 5 training sessions per week and that 1—2 weekly training sessions are sufficient to maintain the better functional capacity thereby achieved which ould otherwise decline to the pre-training level then a couple of months. Saltin (1968) made healthy subjects lie in bed for 3 eeks, and found an impairment in the func tional capacity After the confinement to bed the orking heart rate was higher by an average of 25 beats/min than prior to it, as measured with the same load

The diminished working heart rate is probably due to depressed sympathetic stimulation, and resting bradycardia is consequence of increased agal tone This is suggested by the experiments of Frick et al (1967) using intravenous atropine — propranolol injections. Moreover Tipton (1961) noted in animal experiments that resting bradycardia does not develop after the vagal nerve has been severed. Hakumäki (1970) has proposed that the increase of blood volume due to training results in a better filling of the atria, which increases the number of nerve impulses of atrial origin, thereby slowing down the increase of heart rate.

2. Cardiac output and stroke volume

Christensen (1932) and Frick et al (1963) have noted in groups of both athletes and non-athletes before and after a training period that although the heart rate is low the cardiac output remains at more or less the same level at rest and during a standardized work performance, and that the lower pulse level is compensated for by a greater stroke olume In the above-mentioned study by Saltin et al the heart rate at a constant work load was highest at bed rest, when the stroke volume was smallest. During the training period following bed rest bradycardia developed and the stroke volume increased. The cardiac output did not change. Holmgren et al (1960) Frick et al (1963), Ekblom et al (1968) and Saltin et al (1968) have demonstrated that heavy training effects, in addition to a decline in the heart rate, an increase of the heart size. According to Frick et al (1970) however light training does not necessarily increase the heart size in young people even though the physical working capacity and oxygen intake capacity may increase. The younger one is at the beginning of training, the longer the training lasts, and the more strenuous it is, the greater is the adaptation of the dimensions of the circulatory organs. Sjöstrand (1967) has observed that the increase of stroke volume in young people is accompanied by an increase of the heart size and an increase of the blood volume. Saltin et al (1969) have shown that the training effect can also be attained at an older age, but it is not always possible to increase the heart size. Mann (1969) and Pyörälä et al (1971) however have been able to increase the heart size of even elderly people by training. According to Bevegård et al (1963) and Frick et al (1963) exercise does not seem to affect any changes in the pressures of the systemic circulation and the pulmonary circulation at rest. Bevegård (1963) has noted that during exercise the end-diastolic pressure of the right atrium is higher in trained than in non-trained subjects.

b. Coronary patients

Owing to the laborious methods of investigation and the lack of research materials the data on the

program is slowed down The WHO program has been carried out in practice by Bjurö et al (1971) Thirty two patients out of 117 had to slow down the rehabilitation program because of chest pains, suspicion of reinfarction severe arrhythmia or heart failure. No complications appeared during or immediately after the training sessions, and only a few of the patients felt the training to be too heavy There was no control material in the study The program of Cain consists of 10 activity levels with progressively increasing loads. The last level includes walking up and down stairs like the WHO program The capacity of the patient to take care of himself simultaneously increases step by step Sanne and Selander (1967) examined the effect of Cain s program. They had 84 patients to be rehabilitated and 118 controls. The two groups displayed no differences in complications either during their stay in hospital or one month after the discharge In the study of Helander (1969) both the controls and the patients to be trained were kept in hospital for 21 days, but those undergoing training were exercised progressively from the beginning of the second therapeutic week onwards. The series comprised 250 patients undergoing rehabilitation and 250 controls. The prognosis of 37 patients in each group was followed up for 2 years. No differences in mortality were noted during the follow up

Psychologic effects of training

Black (1956) and Hellerstein et al (1957) have observed that patients are highly disinclined psychologically to exercise themselves after myocardial infarction According to Hellerstein and Goldston (1954) this disinclination is often increased by the caution of the physician in recommending the suitable degree of activity and the tendency of the patient to feel himself a "semi-invalid Martin (1967) has noted that several patients have premorbid depression fatigue distress or anxiety which are inversely related to their attitude towards rehabilitation. According to Wynn (1967) and Wishnie et al (1971) there is a high prevalence of restlessness anxiety and depression after infarction which has a significant influence on the eventual return to work

Hellerstein and Hornsten (1966) found that coronary patients displayed a greater tendency towards depression and psychastenia in psychological tests than healthy subjects. After a training period, the values indicative of depression and psychastenia improved Naughton et al (1968) saw no significant difference in the corresponding test values after active training but noted, however that the patients with training had more regular eating habits, more peaceful sleep and a more tranquil attitude to conflicts at home and in work than those who either trained irregularly or did not train at all. Rechnitzer et al (1967) and McPherson et al (1967) noted that their infarction patients were more tense, aloof taciturn fickle emotionally hurried and aggressive than healthy controls The patients were trained for 24 weeks, after which they were found to be more content and less tense However similar changes were also noted in a control group which gathered for a light recreational swimming session once a week but the changes were more rapid in the training group than in the control group

According to Martin (1967) a close doctor patient relationship is of great importance for the psychic rehabilitation of the patient Miller and Brewer (1969) pointed out that the membership in a training group the activity of this group and the example of others undergoing the same experiences have positive psychologic effects. It is hence difficult to say what role is played by the physical training itself in the psychologic rehabilitation of an infarction patient

Rehabilitation may also have negative psychologic effects. According to Sanne (1973) some infarction patients find that the training sessions remind them of the infarction thereby causing uneasiness and anxiety This is why some people are reluctant to come to hospital for training

Hemodynamic effects of training

The starting point of physical training and the goals set for it vary greatly A competing athlete in top condition is striving towards ever better performances through extreme efforts. A healthy person doing physical exercise is trying to retain his physical and psychic vigour by moving The physical rehabilitation of cardiac patients is often started at the brink of the grave in which case even relatively slight training may clearly improve the functional capacity because the starting level is low The question of how we exercise the circulatory system to improve its condition is not insignificant. Astrand and Rodahl (1970) have shown that dynamic submaximal load of several minutes duration on the large muscle groups increases the aerobic capacity of the organism and hence the type of training which improves the condition of the circulatory system Isometric exercises increase muscular power Maximal loads of short duration increase the anaerobic working capacity but not the aerobic capacity

A. Central hemodynamics

a. He lthy subjects

1 *Heart rate*

In 1931 Christensen was the first to note that endurance training brings down the heart rate of healthy subjects at work. Karvonen et al (1957) point out that in order to reduce the working heart rate, the training heart rate must be greater that the resting heart rate + 60 % of the difference between the maximum rate and the resting rate. For example, maximum heart rate 190 resting heart rate 70 training heart rate ≥ 70 + 60/100 × 120 ≥ 142. Hollman and Venrath (1963), however noted that even an exercise where the heart rate rises up to 115—125/min results in a decline of both the working heart rate and the resting heart rate Åstrand (1960) and Andersen et al (1971) have demonstrated that the maximum heart rate declines along with increasing age. The maximum pulse rate of 20-year-old is appr 200/min and that of a 60-year-old only appr 160/min. Thus, older people reach their maximal aerobic capacity at a lower heart rate, and the training effect is similarly achieved at a relatively lower heart rate. According to Roskamm (1967) 30-minute training session 5 times a eek for one month, at constant 70 % load, brings about maximal decline in the working heart rate measured by the same load. Roskamm maintains that the best increase in functional capacity is reached if the same amount of work is done in 30 minutes, but the load is alternately increased and decreased by 50 % at intervals of 2 1 2 minutes. Saltin (1970) observed that 30-minute training session 2—3 times week brings down the working heart rate nearly as much as 5 training sessions per week, and that 1—2 weekly training sessions are sufficient to maintain the better functional capacity thereby achieved, which would otherwise decline to the pre training level within a couple of months. Saltin (1968) made healthy subjects lie in bed for 3 eeks, and found an impairment in the functional capacity After the confinement to bed the orking heart rate was higher by an average of 25 beats/min than prior to it, as measured ith the same load.

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2. Cardiac output and stroke volume

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b Co onary patients

Owing to the laborious methods of investigation and the limit of research materials, the data on the

effect of training on the hemodynamics of coronary patients are more scarce than the data on the effects noted in the hemodynamics of healthy subjects. In addition to this, the existing results are highly variable and even contradictory

Varnauskas et al (1966) trained 9 physically active coronary patients who were in a good condition on a bicycle ergometer for a month. The patients underwent a 30-minute training session daily on the first week and every second day there after The physical working capacity of all the patients increased. After the training period, the resting heart rate declined significantly the cardiac output did not change while the stroke volume increased significantly After a standardized exercise the working heart rate declined by an average of 6 4 beats/min The cardiac output at the same load was 10 6 % smaller and the stroke volume did not change. Oxygen consumption remained at the same level, but the arteriovenous oxygen difference increased Training reduced the blood lactate concentration during exercise.

Frick et al (1968 and 1970) trained 10 infarction patients. The patients were trained on an ergometer 3 times a week until the occurrence pain or dyspnea. The hemodynamic measurements at rest and at exercise were made in a lying position. With the exception of one patient, the physical working capacity increased in all cases. The heart rate cardiac output and stroke volume did not change at rest The wedge pressure of the pulmonary capillaries and the end-diastolic pressure of the right ventricle increased The working heart rate declined by 10 beats/min the cardiac output did not change and the stroke volume increased. During exercise the systolic pressure of the right ventricle increased slightly No other changes were noted in the pressure measurements. The oxygen consumption and the arteriovenous oxygen differ ence did not change

Clausen et al (1969) trained 9 coronary patients 5 times a week for 4—6 weeks. The load during the training sessions was submaximal at 3—5 minute intervals, and the effective training time was 20—30 minutes. The physical working capacity increased in all cases, by as much as 72 % in one case. The hemodynamic measurements were made in a sitting position. The decline of blood pressure was the only significant change at rest The working heart rate measured at the same load declined by 10 beats/min. The mean arterial pressure declined significantly The cardiac output did not change and the stroke volume increased The blood lactate concentration and TTI declined significantly during exercise The cardiac output of two patients declined significantly during exercise

Physical rehabilitation is accompanied by a placebo effect, as are all therapeutic examinations. Zohman and Tobis (1967) and Bergman and Varnauskas (1971) put forward the idea that the favourable results of training programs might include a significant contributory placebo effect Zohman and Tobis gave 18 angina pectoris patients compressed air instead of oxygen during the placebo period. All the patients felt subjectively better 50 % of them displayed an improved physical working capacity in an exercise test and 20 % had fewer ECG changes than previously The results did not differ from those obtained during a short training period.

The series of Bergman and Varnauskas contained 7 patients with severe angina pectoris and 3 infarction patients. During the placebo period the patients took placebo pills for 4—6 weeks. Four patients felt their condition to be better after the placebo treatment, and only one felt worse than previously The heart rate declined both at rest and at exercise and the pulse rate — blood pressure product declined significantly during work The placebo did not however affect the blood lactate content during exercise, which was lower after the training period According to the authors, this indicates that the training effect is more than a mere placebo effect.

B Peripheral effects

a Healthy subjects

Adaptation due to training also takes place in the circulation of the skeletal muscles and in the musculature itself

Petren et al (1937) and Carrow (1967) have shown that endurance training increases the capillary network of the skeletal muscle in the rat. Hermansen and Wachtlova (1971) propose nevertheless, that such training does not increase the capillaries in man According to Gollnick et al (1973) and Thorstensson (1974) training does not affect the composition of muscle fibers. Gollnick et al (1973) maintain however that heavy endurance training may increase the cross-section area of the slow or red fibers containing abundant myoglobin and mitochondria. Yakovlev (1950) Holloszy (1967) Varnauskas et al (1970) and Kiessling et al (1971) have shown that training increases the size and number of mitochondria in the muscle cells and the activity of respiratory enzymes Therefore changes occur in the muscle cell which cause the oxygen intake to improve

According to Varnauskas et al (1970) and

Clausen and Trap-Jensen (1970) a working muscle manages to do the same amount of work with less blood flow after a training period than previously. Karlsson et al (1970) have noted that the vasoactive metabolites, which reflect muscular perfusion during exercise, are liberated at a greater working load after a training period. Clausen (1970) pointed out that a muscle at exercise is able to increase the blood flow until 70 % of the maximum oxygen intake capacity has been reached, whereafter the increase of flow discontinues. Once this level has been reached, the blood noradrenalin level, which up until then has been only slightly elevated, begins to rise sharply according to Häggendahl et al (1970). Carlsson et al (1968) saw that the noradrenalin content was smaller after a training period than prior to it, when measured with the same working load. The blood noradrenalin originates mainly from peripheral sympathetic nerve endings (Vendsalu 1960). The above agrees with the opinion expressed by Frick et al (1967) concerning the mechanism of bradycardia due to training.

The organism does not improve its capacity until the loading approaches the limit of aerobic functional capacity. Clausen et al (1970) trained the upper extremities of their experimental subjects, but measured the functional capacity before and after training from the lower extremities. No decline in the working heart rate was seen. When the measurements were made on the upper extremities, a clear decline was recorded. The result suggests that bradycardia due to training depends on extracardiac factors.

Rowell et al (1964) and Clausen and Trap-Jensen (1970) have noted that a trained musculature does not utilize an equally great proportion of the cardiac output, after training, as it did before it. The decline of circulation in the splanchnic region and the kidneys at exercise is not equally great after a training period than before it. This change in the regional distribution of blood flow possibly explains why training has no observable effect on the arteriovenous oxygen difference although the oxygen extraction capacity of the musculature increases.

Coronary flow increases somewhat as a consequence of exercise. Penpargkul et al (1970) have noted in animal experiments that a trained heart is capable of a better working performance at rest and during electric pacing than before training. A trained heart consumes more oxygen than a non-trained heart. The additional oxygen required is obtained from the enhanced coronary flow.

b. Coronary patients

Muscular training and changes in peripheral circulation are also contributory factors in the rehabilitation of coronary patients. Clausen et al (1969) noted that muscular flow had declined by 21 % after a training period, when measured with the same submaximal load. Clausen and Trap-Jensen (1970) trained 7 coronary patients for 4—10 weeks. After training the cardiac output was 13.1 % smaller when measured with the same light load, and 5.5 % greater when measured with the same heavy load. At a light load the muscular blood flow declined by 14.9 % while at a heavy load it increased by 8.6 %. The hepatic blood flow did not decline as sharply during exercise after training as it did before.

Detry et al (1971, 1975) have shown that the lower working heart rate after training is compensated by not only a greater stroke volume, but also an increased arteriovenous oxygen difference, which is due to improved oxygen extraction in the muscles.

Effect of training on the coronary circulation of a coronary patient

According to Rose et al (1967), it is possible that intense physical activity may increase the calibre of coronary arteries. Currens and White (1961) found the coronary arteries three times wider than normal at the autopsy of a famous marathon runner who died at the age of 70. It is not known whether training can be used to dilate the coronary arteries of coronary patients.

A heart with coronary sclerosis displays a development of anastomoses (Blumgart et al 1940, Zoll et al 1951, Baroldi et al 1956). Eckstein (1957) and Burt (1965) have demonstrated in dog experiments that after narrowing of the coronary arteries, exercise can be used to bring about collateral formation in the myocardium. Increased collateral circulation would be a natural explanation for the improvement of the cardiac function of a coronary patient in training. According to Levin (1974), however, it has not been possible to prove that training gives rise to collateral formation. Levin (1974) points out, furthermore, that a 90 % coronary narrowing is required before any collateral formation takes place. Franklin (1970) and Webster (1974) have found the collateral formation to have a favourable effect on the prognosis.

Bittar et al (1971) found cineocoronary-arteriographically collateral vasculature in 12 out of their

29 coronary patients. The patients with coronary collaterals did not differ from those without such collaterals with regard to the hemodynamic quantities and the metabolic findings. It thus appears that coronary collaterals are not associated with improved left ventricular performance or metabolism at exercise. Levin (1974) however observed normal left ventricular function to be significantly more frequent in patients with collateral formation than in those without collaterals According to Kattus (1968) the disappearance or decrease of ischemic changes in exercise ECG at a certain heart rate, gives a indirect suggestion of the development of collaterals.

Sarnoff et al (1958) and Sonnenblick (1971) have noted that myocardial oxygen consumption correlated fairly well with the heart rate — blood pressure product if the ejection time of the left ventricle remains constant Robinson (1967) noted that the chest pain of a coronary patient generally appears at the same value of the pulse rate — blood pressure product. If the same work is done at a smaller heart rate and the same or even lower blood pressure after the training period the heart rate — blood pressure product and hence also the myocardial oxygen consumption are smaller Moreover the longer diastole gives the coronary circulation more time Owing to training the coronary circulation thus becomes more economical, even when no collateral formation takes place.

Since the arteriovenous oxygen difference is greater in the coronary circulation than elsewhere in the organism, and since the heart is a continuously functioning muscle it seems unlikely that training could improve the oxygen extraction capacity in the myocardium in the same way as it does in the skeletal muscle. Varnauskas and Holmberg (1971) have noted nevertheless, that coronary patients have a greater oxygen difference than healthy subjects both at rest and at exercise There are indirect demonstrations indicating that exercise would increase coronary circulation or make myocardial enzymatic systems more effective. Katila and Frick (1970) showed that after long term training coronary patients tolerate a greater pulse rate — blood pressure product Redwood et al (1972) have noted that after training coronary patients tolerate a greater triple product (blood pressure × pulse rate × ejection time) before the appearance of chest pains. These findings suggest that the benefit derived from training is not merely of peripheral origin

There is therefore evidence showing that the heart of a coronary patient can tolerate greater loading after a training period So far it is not known whether this is due to increased collateral circulation better and more coordinated contraction or improved myocardial oxygen extraction capacity

Training programs and their effect on functional capacity

The purpose of physical training is to improve the functional capacity of coronary patients and to reduce morbidity and mortality The effective mechanisms are still largely hypothetical. They may be hormonal neurogenic hemodynamic metabolic and emotional. The different effects of physical training can probably be achieved with different mechanisms and the training program should be directed towards them. Since however the mechanisms are imperfectly known the principles of the training programs are fairly arbitrary

The formation of firm scar tissue after infarction takes at least 5 weeks, possibly even 12 weeks, depending on the size of the infarction (Mallory et al 1939 Lodge-Patch 1951) and certain precautions have been observed in starting actual training during the convalescence· intense physical exertion has also been avoided for as long as 3—6 months after the infarction According to the recommendation of the WHO working group (1971) however a submaximal exercise test can be carried out 6—12 weeks after the infarction to estimate the physical functional capacity of the patient as a basis for an individual training program. According to Kellerman (1975) no actual physical training should be started till 8—12 weeks after the infarction because elevated left atrial and left ventricular end-diastolic pressures have been recorded in uncomplicated infarction cases with good recovery as late as 3—8 weeks after the onset of infarction

One of the first comprehensive studies on the training of infarction patients was the work of Newman et al (1952) which covered over 300 patients. There is no control series in the work and no accurate data are given on the results achieved.

Torkelson (1964) trained 10 infarction patients. There was no control series. The training was started in the rehabilitation department of the hospital 2 weeks after the infarction and it was continued for 2 months. An exercise test was performed 6 weeks after the infarction The greatest load used in the training was one where the pulse rate was 10 beats below the maximum The functional capacity of all patients increased The resting and working pulse rates declined and the

ECG changes noted during exercise decreased. Eight of the 10 patients resumed their previous jobs.

Hellerstein (1968) followed up 254 infarction patients for a total of 697 subject years. Rehabilitation was started 3 months after the infarction at the earliest. The patient series was a selected one, for the subjects to be rehabilitated were chosen into the project on the basis of doctors reports. There was no control series. The prognoses were compared with the prognoses of unrehabilitated infarction patients. Each patient had an individual training program based on an ergometer test performed at the beginning of the training period. The objective set for the training was to reach 60—70 % of the maximal aerobic capacity. The training consisted of chalistenics, running periods and ball games. One-hour training sessions were given 3 times week.

During the follow-up period 11 patients under going rehabilitation died, which makes 1 95 patients per 100 subject years. In the patient series used for comparison the corresponding figure was 4.5 — 6.0. The rehabilitation of 100 patients was analyzed in more detail. The average follow-up time was 36 months. Seventy-five percent accomplished the recommended training program. The physical working capacity increased by 21 % measured at a pulse rate of 150/min. The maximal oxygen intake capacity increased from 23.3 to 28.9 ml/kg/min. The pulse rate — blood pressure product at a constant load declined from 284 to 192 $\times$ 10^3 ECG changes decreased by 63 %

The rehabilitation project launched by Gottheiner (1969) in 1955 even included competitive athletics. The training period consisted of 7 classes with progressively heavier exercise. The first 3 classes ere preliminary and the last 4 were actual athletic classes The seventh class included competitions. The patients started the program 10—12 weeks after the infarction. The criterion for selection was the patient own interest. Fifty-five percent of the patients went through all the 7 stages. The drop-out percentage was 40 During follow-up period of 5 years the mortality among the 1103 patients was 3 6 % At the same time the mortality of physically inactive infarction patients in Israel is 12 % The functional capacity increased, the resting and working pulse declined, and the ECG changes decreased during the rehabilitation.

Kellerman and Kariv (1968) have been rehabilitating small groups of infarction patients since 1962, observing the effect of rehabilitation on return to work. Part of the patients were rehabilitated in hospital for 4 months. The patients lived near the hospital, and the bole day was used for physical and occupational rehabilitation. The load in physical training was relatively small, 2—6 cal/min. Another part of the patients had a 45-minute training session 3 times a week. This ambulatory group had more strenuous exercise: 2—9 cal/min. During a 6-year follow-up, the 150 patients rehabilitated had a mortality of 3.3 % while the mortality of the other infarction patients treated in the same hospital was 10 % The functional capacity of the patients rehabilitated in hospital rose from 50 to 72 watts, and that of the ambulatory group from 65 to 93 watts. After the rehabilitation 85 % resumed their previous occupation.

None of the investigations described above had a comparable reference series, which would allow conclusions concerning the effect of rehabilitation on morbidity or return to work or the improvement of the patient's condition in general.

Kentala (1972) divided a series of 298 male infarction patients aged under 65 into a control group and a training group upon admission into hospital. From those who recovered, and subsequently left the hospital, 158 patients were chosen for the actual rehabilitation study which lasted for one year Of those chosen, 81 belonged to the control group and 77 to the training group The first measurement of functional capacity was made 6—8 weeks after the infarction. The training group had a supervised physical training session 3 times a week, where the pulse level was kept about 10 beats below the level reached in the first maximal test for 20 minutes. Functional capacity was measured after 5 and 12 months. Only 33 % of the patients in the training group attended at least 70 % of the supervised training sessions. In contrast the control group increased its physical activity For this reason Kentala re-divided his material according to anamnestic physical activity and the interest in participating in training. The original control and training groups displayed no significant differences in functional capacity in any of the examinations made.

The prognosis was followed up for 2 years. There were no differences in morbidity or mortality Four recidivous infarctions occurred in the control group and 6 in the training group There were 10 coronary deaths in the control group and 8 in the training group. One of the infarctions in the training group occurred during a training session. Nor did supervised training seem to affect the resumption of work. 61 7 % of the control patients and 50 % of the trained patients returned to their earlier occupation within a year The results showed that the physical working capacity of those with

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aged people vary relatively greatly in the different investigations. Grimby et al (1970) obtained 30.5 ml/kg×min as the $\dot{V}o_2$ max of 54-year-old men in Gothenburg. The maximum pulse rate was 172 and the pulse rate at 50 % oxygen intake 108. Siegel (1970) studied the effect of training on nine 32— to 59-year-old blind men with very low activity. Their $\dot{V}o_2$ max was 24 ml/kg×min. In the study by Kasser and Bruce (1969) the $\dot{V}o_2$ max of 117 middle-aged (51 yrs) men was 35.8 ml/kg×min. Kilbom (1971) recorded 31 ml/kg×min as the $\dot{V}o_2$ max for 34- to 48-year-old women and 26.9 ml/kg×min for 51- to 64-year-old women. The degree of heaviness of the work does not seem to have any great effect on $\dot{V}o_2$ max. Bjure et al (1967) recorded a maximum oxygen intake of 30.5 ml/kg×min for construction workers, while Andersen and Hermansen (1965) obtained 36 ml/kg× min for middle-aged men in office work. Andersen and Hermansen (1965) and Grimby and Saltin (1966) have observed that the maximal oxygen intake of middle-aged men active in sports is higher by 40—50 % than that of sedentary men.

Endurance training improves the maximal oxygen intake capacity. Saltin et al (1968) trained 3 untrained young men and noted that a near maximal running exercise 4—5 times a week during 2 months increased $\dot{V}o_2$ max by 33 %. Training 3 times a week gave an almost equally good result when Ekblom (1968) trained untrained people. Roskamm (1967) noted that when the work loads are the same, interval training yields better results than continuous training with lower intensity. Roskamm (1967) further noted that people, who have improved their $\dot{V}o_2$ max by training almost daily do not achieve a positive result with even hard training 3 times a week. In the above mentioned study on blind middle aged men by Siegel (1970) the training was interval training on a bicycle ergometer. The training pulse rate was about 30 beats below the maximum. The oxygen intake capacity increased from 24 to 28.5 ml/kg× min, i.e. by 19 % in 15 weeks. Mann et al (1969) and Saltin et al (1969) made the maximal oxygen intake increase by 15—20 % with maximal or near maximal training, in their works on the trainability of middle-aged and old men. Old people increase their maximal oxygen intake less than middle aged men at the same amount of training and the same initial values. Benestad (1968) saw no rise in the oxygen intake of 70- to 80-year-old men, although their heart rate at a submaximal load decreased after a training period.

Roskamm (1967) found no difference in the trainability of men and women. Six women and 6 men were investigated.

Kilbom (1971) trained healthy women 2—3 times a week for 6—8 weeks. The training session consisted of 30 minutes of interval training on a bicycle ergometer with 18 minutes of active work. Part of the subjects were trained at 70 % intensity, another part at 50 % intensity. The effect of training was followed up in the different age-groups. Maximal oxygen intake increased in all the age-groups during the training period. Before training $\dot{V}o_2$ max was lowest in the oldest group: 19- to 31-year-olds 36.8 ml/kg×min, 34- to 48-year-olds 31.0 ml/kg× min, and 51- to 64-year-olds 26.9 ml/kg×min. The rises in the above groups were 11 %, 13 % and 9 % respectively. Those who were only trained at 50 % intensity had a smaller rise: 6—3 %. At a constant training intensity older women improved their $\dot{V}o_2$ max less than younger. The training effect was smaller in those who initially had a high $\dot{V}o_2$ max. The maximum oxygen intake values of subjects with coronary disease and particularly those with previous myocardial infarction were lower than the corresponding values of healthy subjects of the same age.

Kasser and Bruce (1969) investigated 117 coronary patients, 28 of whom had suffered an infarction, with a mean age of 52 years. Coronary disease was found to diminish the tolerance of maximal exercise, the maximal heart rate, the maximal systolic blood pressure, and the heart rate difference. Systolic blood pressure was 125 mmHg at rest, and 170 mmHg during maximal exercise. Heart rate was 77/min at rest and 142/min during maximal exercise. Diastolic pressure was 84 mmHg at rest and 87 mmHg during exercise. Maximal oxygen intake was 21.9 ml/kg×min, while in a healthy control group of the same age it was 35.8 ml/kg×min. The difference was 39 %.

Benestad (1972) tested 16 male infarction patients with a mean age of 52 years, 12 weeks after the infarction. Maximal oxygen intake was 26.8 ml/kg×min, which is 25 % less than the value *for healthy men of the same age*. The maximum heart rate during maximal exercise was 163/min, which is less than the value for healthy subjects. A low maximal oxygen pulse indicates reduced stroke volume. The findings are therefore indicative of a decline in the pumping capacity of the heart. A decline was also noted in the contractility of the myocardium. The average heart volume was 463 ml/m².

Bruce et al (1973) determined the FAI (functional aerobic capacity) of coronary patients. FAI is defined as the percentage difference between the observed and the expected (age, sex, activity) maximal oxygen intake. FAI was 24 % in a group of men without angina pectoris who had recovered

over 70 % attendance in training increased significantly more than the capacity of the other patients in the training group and that even their initial values were significantly better than those of the others. The physical working capacity of those physically active, regardless of the group increased significantly more than the capacity of the others

Sanne (1973) collected 329 infarctions in Gothenburg during 1968—1970 and 315 of them were included in the research series. When the rehabilitation period began 3 months after the infarction, there were 291 patients 259 of them men and 32 women. The control group consisted of 144 patients, and the training group of 147 Training was started 3 months after the infarction. The aim was to keep the training pulse rate 15 beats lower than the highest working pulse rate recorded in the preceding maximal test. The training program consisted of three 30-minute training sessions weekly The program was of the interval type with 4-minute dynamic periods of chalistenics bicycling jogging running and ball games. A few patients trained at home on a bicycle ergometer according to a strictly defined program 112 of the 148 patients chosen for training started the training program 105 in hospital and 7 at home The drop-out percentage was highest during the first 6 months. After 6 months 52 % continued their training in hospital and 17 % trained at home Two years after the beginning of training only 29 % continued to attend the hospital training In addition to these, 17 % trained at home After 30 months merely 10 % of those who started still came to hospital for training Of those who originally participated in the rehabilitation 64 % attended the training sessions adequately i.e participated in at least 2 training sessions weekly after 3 months and only 53 % did so after 9 months.

One year after the infarction i.e after 9 months of training the patients were re-tested. The control group had the same physical working capacity as it had 3 months after the infarction Those controls, however who were able to bicycle until fatigue had a significantly lower heart rate at the same submaximal load that they had 3 months after the infarction The maximal oxygen intake capacity of these controls was 13 % lower than that of a normal population of the same age Those with symptoms of angina pectoris increased their oxygen intake by 17 %. Those trained subjects who bicycled until fatigue had a significantly greater rise in W max 02 than the controls The trained subjects who stopped bicycling because of angina pectoris has a significant rise in the physical working capacity compared with the controls. After training, the chest pain appeared at a 100 % greater load. The patients who were still training adequately after a year and were able to bicycle until fatigue in the test had increased their maximal aerobic power by 17 % i.e. by the same amount as healthy subjects after training When 2 years had elapsed from the infarction, mortality was significantly higher in the control group. The difference in mortality began to appear 6 months after the infarction There was no difference in recidivous infarctions

All the training programs described above have been carried out in hospitals or rehabilitation centres under the supervision of physiotherapists. There is little experience of supervised self training" at home. Uusitalo and Leskinen (1972) examined the effects of home training in a fairly small series of infarction patients. They divided their series of 41 patients in such a way that half of the patients trained daily at home while the other half came to hospital for a training session supervised by a physiotherapist twice a week Both of the groups were trained according to the rehabilitation program recommended by the WHO working group (1968) There was no inactive control group The follow up time was 2 years. Hospital training had a significantly better effect on the aerobic power and total working capacity than self training The self training physical rehabilitation program however had a positive effect on the submaximal value of aerobic power measured as work load at a heart rate of 150/min. There was no difference between the groups in return to work.

Maximal oxygen intake V_{O_2} max

Maximal oxygen intake (V_{O_2} max) is the product of maximal cardiac output and maximal arterial-mixed venous oxygen difference (Mitchell et al 1958) According to Buskirk and Taylor (1957) it varies with body weight especially lean body mass. In healthy subjects v Döbeln (1956) found men to have higher values than women, but both sexes appeared to have more or less the same V_{O_2} max per kg of body weight, if the body's fat content was disregarded. Young people have higher values than old people, and athletes higher than sedentary persons, even when the differences in body weight are taken into account (Åstrand, P 1962, Astrand, I 1960 Saltin and Åstrand 1967 McDonought et al 1970) According to Saltin et al (1968) V_{O_2} max declines as a consequence of physical inactivity and rises again after training

The oxygen intake values of healthy middle

aged people vary relatively greatly in the different investigations. Grimby et al (1970) obtained 30.5 ml/kg×min as the $\dot{V}_{O_2}$ max of 54-year-old men in Gothenburg. The maximum pulse rate was 172 and the pulse rate at 50 % oxygen intake 108. Siegel (1970) studied the effect of training on nine 32— to 59-year-old blind men with very low activity. Their $\dot{V}_{O_2}$ max was 24 ml/kg×min. In the study by Kasser and Bruce (1969) the $\dot{V}_{O_2}$ max of 117 middle-aged (51 yrs) men was 35.8 ml/kg×min. Kilbom (1971) recorded 31 ml/kg×min as the $\dot{V}_{O_2}$ max for 34- to 48-year-old women and 26.9 ml/kg×min for 51- to 64-year-old women. The degree of heaviness of the work does not seem to have any great effect on $\dot{V}_{O_2}$ max. Bjure et al (1967) recorded a maximum oxygen intake of 30.5 ml/kg×min for construction workers, while Andersen and Hermansen (1965) obtained 36 ml/kg×min for middle-aged men in office work. Andersen and Hermansen (1965) and Grimby and Saltin (1966) have observed that the maximal oxygen intake of middle-aged men active in sports is higher by 40—50 % than that of sedentary men.

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over 70 % attendance in training increased significantly more than the capacity of the other patients in the training group and that even their initial values were significantly better than those of the others The physical working capacity of those physically active regardless of the group increased significantly more than the capacity of the others

Sanne (1973) collected 329 infarctions in Gothenburg during 1968—1970 and 315 of them were included in the research series. When the rehabilitation period began 3 months after the infarction there were 291 patients, 259 of them men and 32 women The control group consisted of 144 patients, and the training group of 147 Training was started 3 months after the infarction The aim was to keep the training pulse rate 15 beats lower than the highest working pulse rate recorded in the preceding maximal test. The training program consisted of three 30-minute training sessions weekly The program was of the interval type with 4-minute dynamic periods of challistenics, bicycling jogging, running and ball games A few patients trained at home on a bicycle ergometer according to a strictly defined program 112 of the 148 patients chosen for training started the training program 105 in hospital and 7 at home. The drop-out percentage was highest during the first 6 months After 6 months 52 % continued their training in hospital, and 17 % trained at home Two years after the beginning of training only 79 % continued to attend the hospital training In addition to these 17 % trained at home After 30 months merely 10 % of those who started still came to hospital for training Of those who originally participated in the rehabilitation 64 % attended the training sessions adequately i.e. participated in at least 2 training sessions weekly after 3 months, and only 53 % did so after 9 months.

One year after the infarction i.e. after 9 months of training the patients were re tested. The control group had the same physical working capacity as it had 3 months after the infarction Those controls, however who were able to bicycle until fatigue had a significantly lower heart rate at the same submaximal load that they had 3 months after the infarction The maximal oxygen intake capacity of these controls was 13 % lower than that of a normal population of the same age Those with symptoms of angina pectoris increased their oxygen intake by 17 % Those trained subjects who bicycled until fatigue had a significantly greater rise in W max 02 than the controls. The trained subjects who stopped bicycling because of angina pectoris has a significant rise in the physical working capacity compared with the controls. After training the chest pain appeared at a 100 % greater load. The patients who were still training adequately after a year and were able to bicycle until fatigue in the test had increased their maximal aerobic power by 17 % i.e. by the same amount as healthy subjects after training When 2 years had elapsed from the infarction mortality was significantly higher in the control group The difference in mortality began to appear 6 months after the infarction There was no difference in recidivous infarctions.

All the training programs described above have been carried out in hospitals or rehabilitation centres under the supervision of physiotheraphists. There is little experience of supervised self training at home. Uusitalo and Leskinen (1972) examined the effects of home training in a fairly small series of infarction patients. They divided their series of 41 patients in such a way that half of the patients trained daily at home while the other half came to hospital for a training session supervised by a physiotherapist twice a week Both of the groups were trained according to the rehabilitation program recommended by the WHO working group (1968) There was no inactive control group The follow up time was 2 years. Hospital training had a significantly better effect on the aerobic power and total working capacity than self training The self training physical rehabilitation program however had a positive effect on the submaximal value of aerobic power measured as work load at a heart rate of 150/min There was no difference between the groups in return to work

Maximal oxygen intake V_{O_2} max

Maximal oxygen intake (V_{O_2} max) is the product of maximal cardiac output and maximal arterial-mixed venous oxygen difference (Mitchell et al 1958) According to Buskirk and Taylor (1957) it varies with body weight, especially lean body mass, in healthy subjects. v Döbeln (1956) found men to have higher values than women but both sexes appeared to have more or less the same V_{O_2} max per kg of body weight if the body's fat content was disregarded. Young people have higher values than old people and athletes higher than sedentary persons, even when the differences in body weight are taken into account (Åstrand P 1962, Åstrand. I 1960 Saltin and Åstrand 1967 McDonough et al 1970) According to Saltin et al (1968) V_{O_2} max declines as a consequence of physical inactivity and rises again after training

The oxygen intake values of healthy middle-

series of Naughton and McCoy (1966) cholesterol declined from 225 mg% to 197 mg% but there was also a significant decline in the control group In the "Gothenburg study (Björntorp et al 1972) the men with infarction, who trained adequately displayed a decline of serum triglycerides and body fat, but no change in serum cholesterol and body cell mass. Plasma insulin declined after a glucose tolerance test, and glucose tolerance increased slightly

There are fewer observations on the effects of training on the serum uric acid content.

Bosco et al (1970), who trained healthy students, noted that heavy training for 8 weeks brought down the serum uric acid content from 6.5 mg% to 5.2 mg% Pyfer and Doane (1969) found no change in the uric acid content when training coronary patients.

Isomäki (1969) noted that coronary patients in Northern Finland had an uric acid content equally as high as hospital patients (5.4 mg%). No correlation between serum cholesterol and uric acid was noted.

Effect of rehabilitation on return to work

There is no complete agreement about the effect of rehabilitation on the resumption of work after infarction. The evaluation of the results is rendered difficult by the small series numbers and the lack of control series in most cases Moreover high values for return to work after myocardial infarction without rehabilitation are reported in the literature.

Honegger (1967) followed up the life of 80 working-age infarction patients after discharge from hospital. There was no rehabilitation. 91 % resumed their previous employment at least partially 71 % became fully capable of performing their previous tasks. A sick leave of 3 months was sufficient for most. Only 17 out of the 80 had a heavy occupation, and 8 of these returned to work.

Shapiro (1971) followed up 470, 25 to 64 year old men who had survived their first infarction by at least 6 months for 4 1/2 years. No rehabilitation was available After the follow-up period 79 % were employed.

Gurevich et al (1971), from the Soviet Union report 57.2 % resumption of work even after the second infarction. Shah (1971) followed up railway officials in Bombay for an average of 24.5 months after the first infarction. Without rehabilitation 75 % returned to their previous employment, and only one out of 116 retired directly

In Scandinavia, Malmcrona et al (1962) and Björk and Wedelin (1964) have reported proportions over 80 % returning to work in unrehabilitated series.

The figures, for spontaneous resumption of work, were also high in Finland before the Sickness Insurance Law came into force. Out of the 242 patients of Iisalo et al (1958) 60.7 % returned to their previous employment, and another 5.6 % took a less strenuous job In Sipilä's (1966) investigation, 62 % of those who survived the infarction, and had been capable of work prior to it, returned to their previous employment, and 17.2 % found a less demanding occupation.

Vuopala (1972) investigated the resumption of work in a group of survivors aged below 65 among the 868 infarction patients treated in the Clinic of Internal Medicine, University Central Hospital of Oulu, during 1 1 1966—30 4 1969 At the onset of the infarction 46.3 % of the men and 41.3 % of the women had been capable of work. 50 % of the men and 31 6 % of the women returned to work in the towns, and 23 4 % of the men and 13 % of the women did so in the country Skilled workers and representatives of the highest social classes returned to work more often than others. The percentage returning to work among the persons with an academic degree was 74 % the corresponding percentage for farmers being 11 9 % and that for smallholders 7 7 % Only three patients found a lighter job 35 % of all those able to work returned to work. The proportion returning to work after the first infarction was 36.6 % among men and 26.3 % among women. None of the women returned to work after the second infarction. None of the men with heavy occupation resumed their previous work after the second infarction.

Siltanen et al (1971) reported that 26.5 % of unrehabilitated infarction patients returned to work in Helsinki.

Kellermann and Kariv (1968) rehabilitated 150 infarction patients and followed them up for 6 years. Altogether 85 % returned to work after rehabilitation. There was no comparable control material in the work. Later on (1975) Kellermann described a series from his hospital, where 91 % of the 299 infarction patients aged under 65 returned to work without rehabilitation.

Of the 10 patients rehabilitated by Torkelson (1964) 8 resumed their previous work. Control material was lacking.

Kentala (1972) found no differences in return to work between trained patients and controls. Of the 81 control patients 50 (61 7 %) resumed their previous employment, while 38 (50 %) patients did so in the group of 77 trained subjects. Out of the patients who had been working before infarction

from infarction and 41 % in a group of men without angina pectoris who had recovered from infarction The maximal oxygen intake value was 26.5 ml/kg × min for the symptomless subjects who had recovered from infarction, and 20 2 ml/kg × min for the subjects who had recovered from infarction, but suffered from angina pectoris.

Training has been shown to elevate the oxygen intake of infarction patients by 20—30 % which means that they reach the same level as untrained people of the same age.

In the rehabilitation study by Hellerstein (1968) mentioned above $\dot{V}O_2$ max rose from 23 2 ml to 28 9 ml/kg × min i e. by about 20 % during a training period. Clausen et al (1969) trained 7 men, 6 of whom had had infarction 1—12 months earlier for 4—6 weeks. As calculated from Åstrand-Ryhming s nomograms, $\dot{V}O_2$ max increased from 24 ml to 30 ml/kg × min, an increase of 30 %

Detry et al (1971) trained 12 coronary patients, 6 of whom had suffered an infarction They trained for 45 minutes, 3 times a week for 3 months. The maximal oxygen intake rose from 23 ml to 28 2 ml/kg × min, a rise of 22.5 % The rise was 30 6 % in patients with angina pectoris, and 17 7 % in the asymptomatic subjects. The bradycardia following training was not compensated for by a greater stroke volume but by an increased A V difference which was due to a higher arterial oxygen content and enhanced peripheral oxygen extraction

Redwood et al (1972) trained 7 coronary patients intensively for 6 weeks After the training the patients tolerated 56 % more exercise before the onset of angina, and at a constant load the angina began 6 8 minutes later The triple product at constant exercise decreased. After the training a greater triple product was achieved before the onset of angina. Training might therefore improve myocardial oxygen delivery The maximal oxygen intake increased significantly during the training period. from 9 6 ml to 15 0 ml/kg × min The average increase was 56 % The exceedingly low initial value which is indicative of the poor functional capacity of the patients is worth emphasising

Metabolic effects of training

When serum lipid values of physically active and inactive subjects have been compared in the different investigations, somewhat contradictory results have been obtained. Shane (1966) observed an inverse correlation between serum cholesterol and physical activity and between serum triglycerides and physical activity but these were not very clear García Palmieri (1972) noted that triglycerides correlated negatively with physical activity while cholesterol did not correlate with physical activity at all. According to Montoye (1969), physically active men had significantly lower cholesterol values than inactive men but there were no differences in glucose tolerance Brunner (1966) found no differences in the cholesterol and triglyceride contents of people doing manual or mental work.

Holloszy et al (1964) demonstrated that when healthy subjects are trained, serum triglycerides decline significantly about 2 hours after strenuous exercise and this decline remains observable for 2—3 days. Holloszy et al (1964) Goode et al (1966) and Siegel et al (1970) have found the decline of triglycerides due to training to be 22—40 % Carlson and Mossfeldt (1964) have recorded a decline of the same order in acute prolonged exercise.

The effect of training on the serum cholesterol level is not equally clear and incontestable. Some authors have noted that training causes a decline in the cholesterol level (Mann 1969 Kilbom 1969), while others have observed no such change (Holloszy 1964 Pyörälä et al 1971)

Johnson et al (1969) noted that both trained and untrained subjects display a similar rise of serum glycerol content during long physical exercise, but the increase of free fatty acids is less pronounced in trained subjects. Their mobilization from the fatty tissue is probably similar but the possibly intensified oxidative metabolism in a trained muscle gives an opportunity for utilizing the fatty acids. The increase in ketones during and after exercise is smaller in trained subjects. According to Cobb et al (1963) the increase of pyruvate and lactate during exercise is smaller in trained subjects. Johnson et al (1969) did not see any change in the glucose tolerance test after a training period.

The results obtained when training coronary patients are also mutually contradictory

According to Varnauskas et al (1966) cholesterol and triglycerides remained at the same level before and after a training period Rechnitzer (1967) also concluded that exercise does not reduce the fat content. Clausen and Trap-Jensen (1970) did not see any change in cholesterol. In the series of Kentala (1972) no changes in serum cholesterol, triglycerides or glucose tolerance were apparent during the follow-up period In Hellerstein s (1969) work cholesterol declined from 263 mg% to 242 mg% during the follow up and in Gehrke s (1967) study it fell from 267 mg% to 206 mg% Neither of the studies had control material and Hellerstein s patients were given dietary instructions. In the

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68.3 % resumed their previous occupation. Social class and physical working capacity were significantly higher among those who returned to work than among the retired ones.

Effect of rehabilitation on the prognosis

There is abundant information on the long-term prognosis after myocardial infarction.

Honey and Truelove (1957) followed up the prognosis of 348 patients who had survived the acute stage of the infarction for 2—16 years. 14 % died during the first year and 10 % during the second year. From the third year onwards mortality was 5 %/year. Cardiac enlargement, arrhythmia and previous infarction were indicative of a poor prognosis.

Zukel et al. (1969) followed up 1 020 infarction patients for 15 years. From the beginning of the second year mortality was 5 %/year, remaining at the same level throughout the whole follow up period.

Pell and D'Alonzo (1964) examined the 5-year prognosis of working-age men with myocardial infarction. Of the 932 patients 74 % were still alive after 5 years.

According to Norris (1970) 4 factors contribute significantly to the prognosis: age, heart volume, degree of pulmonary congestion and ischemic heart disease manifest before the infarction.

In the Oslo study (Myocardial infarction 1956) the lives of 929 infarction patients discharged from hospital were followed up for 18 years. The mortality ratio among 40- to 49 year-old men was 15.5 during the first year, but only 3.7 after 10 years. The corresponding figures for 60- to 69-year-old men were 8.1 and 2.1. The excess mortality following infarction decreased with increasing age. A previous infarction impaired the prognosis. Sievers (1964) investigated the longterm prognosis of 1,589 infarction patients in Malmö who had survived their infarction by 4 weeks. The prognosis was poorer for women than for men. The prognosis for young people compared with a normal population of the same age was poorer than that for old people. The mortality risk of a 40-year-old man with infarction was 6-fold compared with the risk of that age group. The possibility of reinfarction was greater during the first year than later in convalescence. In Holvendahl's (1971) series, the patients with reinfarction had a poorer prognosis than those who had only suffered one infarction.

Although the physical rehabilitation of patients with myocardial infarction has been the object of widespread interest during the last few years, the evaluation of the significance of rehabilitation is rendered difficult by the fact that many of the investigations lack comparable control material. In addition, the follow up periods are relatively short. It is therefore, not yet possible to draw any definite conclusions concerning the effect of physical rehabilitation on morbidity and mortality after myocardial infarction.

In Hellerstein's (1968) series 254 infarction patients were rehabilitated for 697 subject years. The patients were obtained through selection and were in quite good condition. Rehabilitation was started 3 months after the infarction. Among those rehabilitated, 1.95 patients per 100 subject years died. The corresponding figure in the series of coronary patients used in comparison was 4.5—6. Gottheiner (1968) rehabilitated 1 103 voluntary coronary patients. Mortality during a 5-year follow up period was 3.6 %. During the same period the mortality of physically inactive infarction patients in Israel was 12 %.

Kellerman and Kariv (1968) followed the prognosis of 150 rehabilitated infarction patients for 6 years. Mortality during that time was 3.3 %, the mortality of other infarctions treated in that hospital being 10 %. In Kentala's (1972) series the rehabilitation of 74 working age male infarction patients was started 6—8 weeks after the infarction. Rehabilitation was continued for 12 months. There was also a control group of 74 working-age men. Training sessions were first given twice a week, and later 3 times a week. The training consisted of initial warming up followed by calisthenics, rowing and the climbing of stairs. There was no difference in morbidity or mortality between the reference group and the training group during the follow up period. Two years after the beginning of the investigation 6 recidivous infarctions had occurred in the training group and 4 in the control group. Eleven patients had died in each group during that time. There was no difference in overall mortality or coronary deaths between the two groups.

Rechnitzer et al. (1972) have been training infarction patients in Ontario since 1964. Recidivation and mortality were compared with the corresponding values of control material from Toronto in 1969 and local material in 1971. In 1969 the trainees had been active for at least 3 months, in 1971 for at least one year. In each study both recidivation and coronary mortality were significantly lower in the trained group. In 1969 mortality was 3.9 % in the trained group and 11.8 % in the control group, while recidivous infarctions occurred in 1.3 % of the cases in the trained group and in 27.9 % of the control cases.

In 1971 mortality was 7.6 % in the trained group and 18.9 % in the control group; recidivation was 3.0 % in the trained group and 11.1 % in the control group. The control series used in these studies were however somewhat inadequate. In 1969 the control series was from a different locality and the criteria for recidivous infarction were not the same for the controls and the trained subjects. In 1971 the control group consisted of the patients unwilling to attend rehabilitation who may have had a poorer starting point than the trained subjects.

In the Gothenburg study (Sanne et al 1971) where 111 of the 156 subjects chosen for rehabilitation started training and where there was an identical control series of 153 patients, coronary deaths were significantly fewer in the training group (6) than in the control group (18) during the period between the 26th and the 140th week of training. The groups did not differ in the occurrence of recidivous infarctions. However in the same material, after four years follow-up a significant positive effect of physical training on mortality or reinfarction rate could not be shown (Wilhelmsen et al 1976).

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training group contained some more women capable of work than the control group: 27 (73 %) and 15 (44 %). The difference was not significant (Table VII).

The reasons for disability did not differ in the different groups. Appr 75 % of the men and appr 90 % of the women were unable to work because of a coronary disease (Table VIII)

Most of the subjects had now suffered their first infarction (Table X). The groups did not differ as regards the number of infarctions suffered. Nor were there any differences in the previous symptoms. Over 70 % of the subjects in all the groups had suffered from chest pain or chest pain and dyspnea prior to infarction. Appr 20 % of all men and appr 10 % of all women had been completely asymptomatic (Table XI).

The duration of the preceding symptoms was not different in the male training group and the male control group (Table XII). The female training group included 10 (29.5 %) women who had had symptoms for less than a year while only one (3.2 %) such woman was found in the control group Correspondingly there were slightly more women with long term symptoms in the control group The differences were not, however significant. The time which elapsed before admission into hospital was not different in the different groups (Table XIII)

The groups did not differ as regards the severity of infarction of the complications (Tables XIV and XV) Appr 60 % of the men and appr 50 % of the women had a transmural infarction.

Complicated infarctions (resuscitation, serious arrhythmia, II—III AV block, pulmonary oedema, prolonged chest pains) were suffered by 15 (10.5 %) training men, 23 (13 9 %) control men, 7 (18.9 %) training women and 5 (14 7 %) control women.

The groups did not differ as regards the localization of the infarction (Table XVI). Anterior infarctions were noted in 77 (53 9 %) training men 88 (53.0 %) control men, 26 (70.3 %) training women and 25 (73.5 %) control women. Among the subjects with anterior infarctions, men had more anteroseptal ones than women (Table XVII).

Material

The 380 patients, who made up the total material suffered a myocardial infarction during the period May 1 1969 — Aug 17 1971 All these patients were treated in the Clinic of Internal Medicine, University Central Hospital of Oulu. The following criteria were used in diagnosing myocardial infarction.

— chest pain,
— Q (transmural infarction) or ST and T (intramural infarction) changes in the ECG
— elevation of the ASAT and/or HBD values,
— leukocytosis.

In addition to the ECG changes, at least two other positive findings were required. In a few cases, with branch blocks, the infarction was diagnosed on the basis of three other positive criteria.

Four weeks after discharge (6 weeks after the onset of infarction) the patients came for a checkup in an outpatient department. In this checkup each patient was distributed into either the training group or the control group Men and women aged under 65 i e. working age subjects, were accepted into the series. Incapacity to work prior to infarction was no obstacle for being accepted. Subjects with severe motor invalidity (hemiplegia amputation of the lower extremities) and psychiatric cases with uncertain cooperation were excluded from the series. Those with severe decompensated heart failure (resting dyspnea, a large heart and/or pulmonary oedema in the roentgenogram) diagnosed in the checkup were similarly excluded. A few patients refused to participate because of the distance and/or difficulty of transportation between home and hospital. The division of the subjects into a training group and a control group was accomplished by taking the first 100 into the training group and the next 100 into the control group Since it appeared that almost exactly 200 patients were obtained annually the division was continued by taking subsequent groups of only 50 subjects, to eliminate seasonal variation Altogether 200 training subjects and 200 control subjects were collected In a subsequent examination of the material 20 patients had to be left out of the first training group because of excessive age or uncertain diagnosis (pre-infarctional angina) The final material, thus consisted of 180 training subjects 143 men and 37 women, and 200 controls, 166 men and 34 women (Table I)

The drug therapy was the same for the training group and the control group Heart failure was treated with digoxin and diuretics. The patients with angina pectoris were given nitroglycerine preparations. So-called coronary dilatators and beta blocking agents were not used.

The mean age of the training men was 52.4 yrs and that of the control men 51 6 yrs. The training women had a mean age of 54 7 yrs and the control women 57 4 yrs. The differences were not significant (Table II) The training and the control groups did not differ as regards marital status (Table III)

Out of the men in the training group 62 (43 4 %) lived in the country and 81 (56.6 %) in town. Of the control men 100 (60 2 %) lived in the country and 66 (39.8 %) in town (Table IV) Thus, the majority of the training men were urban dwellers and a majority of the control men rural dwellers. The difference was highly significant. The training women and the control women did not differ as regards the place of residence.

The occupational distribution did not differ in the groups of training men and control men (Table V) Among the training women 10 (27 0 %) were unskilled, while the female control group only contained one (2.9 %) unskilled subject. The difference was almost significant. The group of control women included more urban housewives and wives of farmers than the female training group 25 (73.5 %) and 17 (45 9 %)

There were slightly more men doing heavy manual work in the male control group than among the training men. 59 (35.5 %) and 38 (26.6 %) (Table VI)

The groups did not differ from each other in leisure time physical activity prior to infarction (Table IX) Appr 70 % of the men and appr 80 % of the women had not engaged in any physical activity Activities with a significant training effect (jogging, bicycling, swimming, ball games etc 3 hrs/week) had only been undertaken by 8 men 2 in the training group and 6 in the control group

Disability to work at the onset of infarction was not different in the two male groups The female

blocks, entricular blocks, atrial fibrillation and extrasystoles. The thorax roentgenograms were analyzed for the size of the heart, hypertrophies and pulmonary congestion

The physician was present throughout the 1st—6th chalistenic sessions as well as the 9th and 12th sessions. The 7th, 8th 10th and 11th sessions were teaching sessions supervised by a physiotherapist. The gymnastic hall had the facilities and equipment needed for resuscitation. ECG monitoring was not available, but the subject taking the ergometric test as under auscultatory control. In practice the test subject as auscultated for appr 3/4 of the testing time ith only the brief conversations concerning the subject' condition interrupting the auscultation. If several extrasystoles were heard in the auscultation, the ergometric test was interrupted. The test bicycle as a Monark cyclometer The same bicycle was used throughout the test; its calibration was checked monthly but the calibration did not need to be changed

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Three, 6, 9 and 12 months after the initial checkup the serum cholesterol, triglycerides and uric acid ere determined and 2 hour glucose tolerance test was performed. Cholesterol was determined according to the calorimetric method (Pearson et al 1953) triglycerides by an LKB-Auto-Analyser (Boehringer's package Eggstein 1966) urate by the enzymatic method t 37° C (Liddle et al 1959), and glucose by the glucose oxidase method at 37° C (Huggett et al 1957). Glucose was administered in a dose of one gram per kg of body weight. In addition to the fasting value, the values at 1 hour and 2 hours were obtained.

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Training program:

Early mobilization was aimed at in the hospital therapy. The patients with an intramural infarction were kept in bed for 3 days. After a week spent in a sitting position, the patients were ordered to walk on the 10th day and were discharged on the 12th day. The patients with a transmural infarction were kept in bed for 7 days. After a week of sitting they were ordered to walk on the 14th day and discharged on the 16th day. In complicated cases the patients were kept in bed and hospital for as long as the complication required.

One month after the checkup in an outpatient department the subjects to be rehabilitated were invited to the first training session, during which they took their first test on a bicycle ergometer. The training took place in a gymnastic hall in groups of 5—7 subjects. A physiotherapist conducted the exercises, and the whole session was supervised by a physician. The instructions of the WHO working group (1968) were followed. The training was started with breathing and relaxation movements, which were followed by series of various chalistenic movements involving all the muscle groups of the extremities and the body. The training also included walking on the spot, and later running on the spot. The objective of this interval training technique is a degree of strain which brings up the pulse rate of the trainers to at least 70 % of the maximum pulse rate of their age group.

As the rehabilitation proceeded, the amount of exercise was progressively increased, e.g. by including the use of small hand weights in the program. After each session the trainers were given a duplicate of the program learnt, to be taken home. The program lasted for about 30 minutes, and the patients were instructed to perform it at home every day. They were also asked to make notes of their chalistenic activity and the time spent in chalistenics. After a month's training at home the subjects came for the next supervised session, during which they were taught new, somewhat more strenuous series of movements. An ergometric test was performed on the same occasion. The groups thus underwent a supervised 30-minute session in a gymnastic hall once a month, and between these sessions they trained at home, making notes of their own activity.

The bicycle ergometer test was made once a month until the 6th month. Thereafter the training groups were tested every 3rd month. The training was continued for 12 months. After the 3rd, 6th, 9th and 12th training session the subjects underwent a checkup in an outpatient department. In these checkups anamnestic information was sought for the frequency of chest pains (no pains, occasional pains, monthly, weekly or daily pains), the cause of the chest pains (use of hands, walking, excitement, pain while sleeping, other), the duration of the pains (min, half an hour, hours) and the grade of dyspnea (I—IV) (Rose and Blackburn 1968). The free time physical activity other than chalistenics was also graded according to Wilhelmsen et al (1971) (sedentary, walking 4 hrs/week, running etc. 3 hrs/week, active exercise several times a week) and the smoking habits were recorded (non smoker, ex smoker, gave up smoking at the onset of infarction, smoker, the amount of tobacco consumed in g/day).

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from nomograms constructed according to Du Bois and Du Bois (1916) Further estimates made from the roentgenogram included left ventricular hyper trophy left atrial hypertrophy increase of pulmonary congestion (PV+) and interstitial oedema these parameters were estimated as in conventional clinical practice.

The electrocardiograms were obtained with a Mingograf -34 a three-channel ECG apparatus.

Statistics:

The significanses and standard deviations of the means were calculated by Student s t test, using the following formula. (Woolf C M. 1968)

$$t = \left| \frac{\bar{x} - \bar{y}}{\sqrt{\frac{1}{n_x} + \frac{1}{n_y}} \sqrt{\frac{\Sigma (x_i - \bar{x})^2 + \Sigma (y - \bar{y})^2}{n + n_y - 2}}} \right|$$

When calculating the significance of the changes which took place in each patient group during the follow up period, the paired t test was used, and only those patients were compared who remained in the research series throughout the follow-up The means were, however always calculated for all those who attended the checkup in question.

The frequency differences were tested by the chi-square test. The significance was determined as follows: $p < 0\ 05$ was almost significant, $p < 0.01$ was significant, and $p < 0\ 001$ was highly significant.

Results

Feasibility of physical rehabilitation

Participation in supervised challstenic sessions and checkups in the outpatient department

Three months after the initiation of supervised training, 78 % of the training men and 62 % of the training women were attending the supervised monthly challstenic sessions. The session of the 6th month was still attended by 78 % of the men and 65 % of the women. 67 % of the men and 62 % of the women were present in the last session on the 12th month. There were no differences between the groups in their attendance at the checkups made in an outpatient department. The checkup of the 3rd month was made on 94 % of the training men, 91 % of the control men, 95 % of the training women and 100 % of the control women. After 6 months 89 % of the training men, 87 % of the control men, 87 % of the training women and 94 % of the control women visited the outpatient department. Those present in the last checkup included 85 % of the training men, 84 % of the control men, 76 % of the training women and 85 % of the control women (Table XVIII).

Challstenic activity at home

Throughout the follow-up year more than half of both the men and the women reported that they did challstenic exercises at least 6 days of the week. At the time of the 6-month checkup 55 % of the training men and 70 % of the training women did challstenic exercises on 6—7 days of the week, while the corresponding percentages at the 12-month checkup were 51 % for men and 73 % for women. For 12 % of the men and 17 % of the women the amount of challstenic exercises performed was insignificantly small, i.e. 0—2 times a week, at the time of the 6-month checkup, and 15 % of the men and 14 % of the women had come down to this level by the 12-month checkup (Table XIX).

These training men and training women, who did challstenics at home, spent an average of over half an hour in each training spell, throughout the entire follow-up year (Table XX).

The skill of the participants in supervised challstenic sessions was estimated on the basis of how well they were able to perform the movements presented the previous time. The skill was graded on a 1—3 scale. The purpose of this was to check the challstenic activity. With a few exceptions, the trainers were able to perform their movements well (Table XXI). The reports of the patients concerning their challstenic activity were therefore probably truthful.

Anamnestic data

Subjective symptoms

At the time of the initial checkup 18.9 % of the training men, 15.7 % of the control men, 18.9 % of the training women and 11.8 % of the control women were without symptoms of chest pain (Table XXII). The patients with daily symptoms at that time accounted for 28 % of the training men, 31.9 % of the control men, 24.2 % of the training women and 29.4 % of the control women. At the end of the follow-up period the proportion of painless subjects was smaller in all groups: 13.2 % of the training men, 13.7 % of the control men, 14.3 % of the training women and 10.3 % of the control women. In none of the groups was the change significant. The proportion of subjects with daily pains was also smaller in all groups after 12 months than initially: 22.3 % of the training men, 27.3 % of the control men, 17.9 % of the training women and 27.6 % of the control women. The change was not significant in any group. There were no significant differences in the frequency of pains between the training men and the control men or the training women and the control women at any checkup, nor did any significant change in the occurrence of pains take place within any group.

At the initial checkup 62.1 % of the training men, 67.1 % of the control men, 63.3 % of the training women and 77 % of the control women reported use

of hands as the cause of chest pain (Table XXIII) At the 3-month checkup the number of subjects suffering from chest pain due to use of hands was greater in all the groups After a year the use of hands brought about chest pain in 77 1 % of the training men (compared with the initial checkup $p < 0\ 05$) 89 2 % of the control men ($p < 0\ 001$) 87.5 % of the training women ($< 0\ 05$) and 80 8 % of the control women (no significant difference compared with the initial checkup) A significantly greater number of the control men ($p < 0\ 01$) suffered from chest pain due to use of hands at the 9-month checkup compared with the training men and an almost significantly greater number ($p < 0\ 05$) did so at the 12 month checkup None of the checkups revealed any significant differences in the pains due to use of hands by the training women and the control women.

The occurrence of chest pains due to walking and excitement did not change significantly in any patient group during the follow up year and no differences between the groups were noted at the checkups.

Both the training and the control men reported more chest pains in sleep at the 12 month checkup than initially ($p < 0\ 05$) At the 3-month checkup the control men had almost significantly ($p < 0\ 05$) more pains in sleep than the training men The women experienced no change in the night time pains during the follow up and the female groups displayed no differences at any checkup

At the time of the initial checkup 11 2 % of the training men 12.8 % of the control men 13 2 % of the training women and 10 % of the control women suffered from prolonged chest pains (Table XXIV) The duration of the pains did not change in the female groups during the year and no differences appeared between the groups The training men similarly experienced no change in the duration of chest pains but in the group of control men the occurrence of prolonged chest pains increased. At 9 months 26.5 % of the control men had prolonged chest pains which was a significantly greater proportion than initially ($p < 0\ 01$) At the 9 month checkup 15 1 % of the training men had prolonged chest pains, the difference between them and the controls being almost significant ($p < 0\ 05$) At all the other checkups, there were also fewer training men than control men suffering from prolonged chest pains but the differences were not significant

At the time of the initial checkup 63 6 % of the training men, 56 % of the control men, 43 2 % of the training women and 35 3 % of the control women were without symptoms of dyspnea (Table XXV) The dyspneic symptoms increased in all the four groups during the follow up period and hence only 49 6 % of the training men, 41 % of the control men 35 7 % of the training women and 27 6 % of the control women were without dyspneic symptoms at the end of the follow up year In the group of training men the change was almost significant ($p < 0\ 05$) and in the group of control men significant ($p < 0.01$). The changes in the female groups were not significant None of the checkups revealed any differences in the grade of dyspnea between the training women and the control women.

Physical activity in leisure time manner of training (Tables IX and XXVI)

The training groups and the control groups did not differ with regard to leisure time physical activity before the onset of infarction 67.8 % of the training men and 68.1 % of the control men were sedentary while 30 8 % of the training men and 28.3 % of the control men did some walking Only 2 of the training men and 6 of the control men had been doing physical activity with a significant training effect (jogging biking swimming, ball games etc 3 hours/week) prior to their infarction. In the groups of both training women and control women appr 80 % were sedentary and none of the women had participated in any training activity

At the time of the initial checkup 20.3 % of the training men and 32.5 % of the control men walked for at least 4 hrs/week At the initial checkup 97 % of both the female groups were sedentary

All the other groups except that of control women increased their leisure time activity during the follow up year After the year 25 6 % of the training men 38 8 % of the control men 57 1 % of the training women and 79 3 of the control women were sedentary

Both the training men and the control men were significantly more active physically at the time of the 3-month checkup than they had been prior to their infarction ($p < 0\ 01$) and the physical activity of both groups continued to increase until the end of the year Although the training men were somewhat more active physically than the controls, no statistically significant differences in leisure time physical activity appeared between the groups during the follow up year Neither was there any difference in leisure time physical activity between the training women and the control women at any checkup The training women were however more active than the controls, and clearly increased their physical activity compared with the pre infarctional time ($p < 0\ 05$)

Fatigue, claudication (Table XXVII)

Subjective fatigue decreased in all the four patient groups during the follow-up year. Among the training men and control women the change was highly significant ($p < 0.001$), among the control men significant ($p < 0.01$) and among the training women almost significant ($p < 0.05$). At the 9-month checkup 25.2 % of the training men and 37.6 % of the control men complained of fatigue; this difference was almost significant ($p < 0.05$) but no other checkup revealed any differences between the training men and the control men or the training women and the control women.

The symptoms of claudication did not change significantly in any of the patient groups during the year and no significant difference was noted between the training women and the control women at any checkup. At the time of the initial checkup 12.6 % of the training men and 21.1 % of the control men had symptoms of claudication, the difference not being significant. The training men displayed significantly less claudication at 3 and 6 months than the controls and almost significantly less claudication after 9 and 12 months. At the 12-month checkup 18.2 % of the training men and 30.2 % of the controls had claudication.

The claudication distance did not change significantly in any of the patient groups during the year and no checkup revealed any differences between the training men and the control men, or the training women and the control women (Table XXVIII).

Smoking (Tables XXIX and XXX)

No effort was made to influence the smoking habits of the patients.

At the time of the initial checkup 33.6 % of the training men, 36.1 % of the control men, 16.2 % of the training women and 5.9 % of the control women smoked. After having suffered an infarction, 39 training men (27.4 %), 48 control men (28.9 %), 7 training women (18.9 %) and 6 control women (17.7 %) had given up smoking. During the follow-up year 12 training men, 14 control men, 2 training women and 4 control women resumed smoking. Thus 35 % of the training men, 41 % of the control men, 17.9 % of the training women and 17.2 % of the control women smoked at the end of the year. Smoking did not change significantly in any group during the follow-up year and none of the checkups revealed any differences in smoking between the training and the control groups.

The amount of tobacco consumed was not different in the training and the control groups at any stage, and the amount consumed did not change within any group during the follow-up.

Clinical findings

Inspection, palpation and auscultation of the heart revealed only one almost significant difference between the training men and the control men during the whole follow-up year: at the 12-month checkup the training men had more MR sounds than the controls ($p < 0.05$). The training men had more MR sounds than the control men at the initial checkup as well as at all the others, but these differences were not significant (Table XXXI).

The auscultation of both the training men and the control men at the 12-month checkup revealed more ($p < 0.01$) S4 gallops than at the initial checkup. No change was observable in the frequency of S3 gallops in either of the male groups. None of the examinations revealed any difference in the frequency of the gallop sounds between the training men and the control men. The control women had almost significantly more ($p < 0.05$) S4 gallops than the training women at the initial checkup and at 3 and 12 months (Table XXXII). No other differences were noted in the cardiac status of the training women and the control women.

Neither the training men and the control men, nor the training women and the control women differed in mean body weight at any time during the follow-up (Table XXXIII).

The mean body weight of the control men was highly significantly ($p < 0.001$) greater at the 3-month checkup and significantly ($p < 0.01$) greater at 6 and 9 months than initially. The mean weight of the training women was significantly ($p < 0.01$) lower at 9 and 12 months than initially.

By the end of the follow-up year 50 (41 %) training men, 67 (48.2 %) control men, 5 (16.7 %) training women and 11 (38 %) control women had gained weight, while 59 (48.3 %) training men, 51 (36.7 %) control men, 20 (66.7 %) training women and 18 (62 %) control women had lost weight. The individual weight variations were not significantly different in the training groups and the control groups.

None of the checkups showed any difference in the mean systolic blood pressure between the training men and the control men or the training women and the control women (Table XXXIV). The mean systolic blood pressure of the training men declined, being significantly lower at 6 and 9

of hands as the cause of chest pain (Table XXIII) At the 3-month checkup the number of subjects suffering from chest pain due to use of hands was greater in all the groups. After a year the use of hands brought about chest pain in 77 1 % of the training men (compared with the initial checkup $p < 0.05$) 89 2 % of the control men ($p < 0.001$) 87.5 % of the training women (< 0.05) and 80 8 % of the control women (no significant difference compared with the initial checkup) A significantly greater number of the control men ($p < 0.01$) suffered from chest pain due to use of hands at the 9 month checkup compared with the training men, and an almost significantly greater number ($p < 0.05$) did so at the 12 month checkup None of the checkups revealed any significant differences in the pains due to use of hands by the training women and the control women

The occurrence of chest pains due to walking and excitement did not change significantly in any patient group during the follow up year and no differences between the groups were noted at the checkups.

Both the training and the control men reported more chest pains in sleep at the 12 month checkup than initially ($p < 0.05$) At the 3-month checkup the control men had almost significantly ($p < 0.05$) more pains in sleep than the training men The women experienced no change in the night time pains during the follow up and the female groups displayed no differences at any checkup

At the time of the initial checkup 11 2 % of the training men, 12.8 % of the control men 13.2 % of the training women and 10 % of the control women suffered from prolonged chest pains (Table XXIV) The duration of the pains did not change in the female groups during the year and no differences appeared between the groups. The training men similarly experienced no change in the duration of chest pains, but in the group of control men the occurrence of prolonged chest pains increased At 9 months 26 5 % of the control men had prolonged chest pains which was a significantly greater proportion than initially ($p < 0.01$) At the 9 month checkup 15 1 % of the training men had prolonged chest pains, the difference between them and the controls being almost significant ($p < 0.05$) At all the other checkups there were also fewer training men than control men suffering from prolonged chest pains but the differences were not significant

At the time of the initial checkup 63 6 % of the training men 56 % of the control men 43 2 % of the training women and 35.3 % of the control women were without symptoms of dyspnea (Table XXV) The dyspneic symptoms increased in all the four groups during the follow up period, and hence only 49 6 % of the training men 41 % of the control men 35 7 % of the training women and 27 6 % of the control women were without dyspneic symptoms at the end of the follow up year In the group of training men the change was almost significant ($p < 0.05$) and in the group of control men significant ($p < 0.01$) The changes in the female groups were not significant None of the checkups revealed any differences in the grade of dyspnea between the training women and the control women

Physical activity in leisure time manner of training (Tables IX and XXVI)

The training groups and the control groups did not differ with regard to leisure time physical activity before the onset of infarction. 67 8 % of the training men and 68 1 % of the control men were sedentary while 30 8 % of the training men and 28.3 % of the control men did some walking Only 2 of the training men and 6 of the control men had been doing physical activity with a significant training effect (jogging biking swimming ball games etc 3 hours/week) prior to their infarction In the groups of both training women and control women appr 80 % were sedentary and none of the women had participated in any training activity

At the time of the initial checkup 20.3 % of the training men and 32.5 % of the control men walked for at least 4 hrs/week. At the initial checkup 97 % of both the female groups were sedentary

All the other groups except that of control women increased their leisure time activity during the follow up year After the year 25 6 % of the training men, 38 8 % of the control men 57 1 % of the training women and 79 3 of the control women were sedentary

Both the training men and the control men were significantly more active physically at the time of the 3-month checkup than they had been prior to their infarction ($p < 0.01$) and the physical activity of both groups continued to increase until the end of the year Although the training men were somewhat more active physically than the controls, no statistically significant differences in leisure time physical activity appeared between the groups during the follow up year Neither was there any difference in leisure time physical activity between the training women and the control women at any checkup The training women were however more active than the controls, and clearly increased their physical activity compared with the pre infarctional time ($p < 0.05$)

Fatigue, claudication (Table XXVII)

Subjective fatigue decreased in all the four patient groups during the follow-up year. Among the training men and control women the change was highly significant ($p < 0.001$), among the control men significant ($p < 0.01$) and among the training women almost significant ($p < 0.05$). At the 9-month checkup 25.2 % of the training men and 37.6 % of the control men complained of fatigue; this difference was almost significant ($p < 0.05$), but no other checkup revealed any differences between the training men and the control men or the training women and the control women.

The symptoms of claudication did not change significantly in any of the patient groups during the year and no significant difference was noted between the training women and the control women at any checkup. At the time of the initial checkup 12.6 % of the training men and 21.1 % of the control men had symptoms of claudication, the difference not being significant. The training men displayed significantly less claudication at 3 and 6 months than the controls and almost significantly less claudication after 9 and 12 months. At the 12-month checkup 18.2 % of the training men and 30.2 % of the controls had claudication.

The claudication distance did not change significantly in any of the patient groups during the year and no checkup revealed any differences between the training men and the control men, or the training women and the control women (Table XXVIII).

Smoking (Tables XXIX and XXX)

No effort was made to influence the smoking habits of the patients.

At the time of the initial checkup, 33.6 % of the training men, 36.1 % of the control men, 16.2 % of the training women and 5.9 % of the control women smoked. After having suffered an infarction 39 training men (27.4 %), 48 control men (28.9 %), training women (18.9 %) and 6 control women (17.7 %) had given up smoking. During the follow-up year 12 training men, 14 control men, 2 training women and 4 control women resumed smoking. Thus 38 % of the training men, 41 % of the control men, 17.9 % of the training women and 17.2 % of the control women smoked at the end of the year. Smoking did not change significantly in any group during the follow-up year and none of the checkups revealed any differences in smoking between the training and the control groups.

The amount of tobacco consumed was not different in the training and the control groups at any stage, and the amount consumed did not change within any group during the follow-up.

Clinical findings

Inspection, palpation and auscultation of the heart revealed only one almost significant difference between the training men and the control men during the whole follow-up year: at the 12-month checkup the training men had more MR sounds than the controls ($p < 0.05$). The training men had more MR sounds than the control men at the initial checkup as well as all the others, but these differences were not significant (Table XXXI).

The auscultation of both the training men and the control men at the 12-month checkup revealed more ($p < 0.01$) S4 gallops than at the initial checkup. No change was observable in the frequency of S3 gallops in either of the male groups. None of the examinations revealed any difference in the frequency of the gallop sounds between the training men and the control men. The control women had almost significantly more ($p < 0.05$) S4 gallops than the training women at the initial checkup and at 3 and 12 months (Table XXXII). No other differences were noted in the cardiac status of the training women and the control women.

Neither the training men and the control men, nor the training women and the control women differed in mean body weight at any time during the follow-up (Table XXXIII).

The mean body weight of the control men was highly significantly ($p < 0.001$) greater at the 3-month checkup and significantly ($p < 0.01$) greater at 6 and 9 months than initially. The mean weight of the training women was significantly ($p < 0.01$) lower at 9 and 12 months than initially.

By the end of the follow-up year 50 (41 %) training men, 67 (48.2 %) control men, 5 (16. %) training women and 11 (38 %) control women had gained weight, while 59 (48.3 %) training men, 51 (36.7 %) control men, 20 (66.7 %) training women and 18 (62 %) control women had lost weight. The individual weight variations were not significantly different in the training groups and the control groups.

None of the checkups showed any difference in the mean systolic blood pressure between the training men and the control men or the training women and the control women (Table XXXIV). The mean systolic blood pressure of the training men declined, being significantly lower at 6 and 9

of hands as the cause of chest pain (Table XXIII) At the 3-month checkup the number of subjects *suffering from* chest pain due to use of hands was *greater in all the groups. After* a year the use of *hands brought about* chest pain in 77 1 % of the training men (compared with the initial checkup $p < 0.05$) *89.2 % of the* control men ($p < 0.001$) 87.5 % of the training women (< 0.05) and 80 8 % of the control *women (no significant difference* compared with the initial checkup) A significantly greater *number of the control men* ($p < 0.01$) suffered from chest pain due to use of hands at the 9 month checkup *compared with the training* men and an almost significantly greater number ($p < 0.05$) did so at the 12 month checkup *None* of the checkups revealed any significant differences in the pains due to use of hands by the training women and the control women.

The occurrence of chest pains due to walking and excitement did not change significantly in any patient group during the follow up year and no differences between the groups were noted at the checkups.

Both the training and the control men reported more chest pains in sleep at the 12 month checkup than initially ($p < 0.05$) At the 3-month checkup the control men had almost significantly ($p < 0.05$) more pains in sleep than the training men The women experienced no change in the night time pains during the follow up and the female groups displayed no differences at any checkup

At the time of the initial checkup 11 2 % of the training men 12.8 % of the control men 13 2 % of the training women and 10 % of the control women suffered from prolonged chest pains (Table *XXIV) The duration of the pains did not change* in the female groups during the year and no *differences appeared between the groups.* The training men similarly experienced no change in the *duration of chest pains, but in the group of control men the occurrence of prolonged chest pains increased* At 9 *months* 26.5 % of the control men had prolonged chest pains, which was a significantly greater proportion than initially ($p < 0.01$) At the 9-month checkup 15 1 % of the training men had prolonged chest pains, *the difference* between them and the controls being almost significant ($p < 0.05$) At all the other checkups, there were also fewer training men than control men suffering from prolonged chest pains but the differences were not significant

At the time of the initial checkup 63 6 % of the *training men* 56 % *of the* control men 43 2 % of the training women and 35 3 % of the control *women were without* symptoms of dyspnea (Table XXV) The dyspneic symptoms increased in all the four groups during the follow up period and hence only 49 6 % of the training men 41 % of the control men, 35 7 % of the training women and 27 6 % of the control women were without dyspneic symptoms at the end of the follow up year In the group of training men the change was almost significant ($p < 0.05$) and in the group of control men significant ($p < 0.01$) The changes in the *female groups* were not significant None of the checkups revealed any differences in the grade of dyspnea between the training women and the control women

Physical activity in leisure time manner of training (Tables IX and XXVI)

The training groups and the control groups did not differ with regard to leisure time physical activity before the onset of infarction 67 8 % of the training men and 68 1 % of the control men were sedentary while 30 8 % of the training men and 28.3 % of the control men did some walking Only 2 of the training men and 6 of the control men had been doing physical activity with a significant training effect (jogging, biking swimming, ball games etc 3 hours/week) prior to their infarction. In the groups of both training women and control women appr 80 % were sedentary and none of the women had participated in any training activity

At the time of the initial checkup 20.3 % of the training men and 32.5 % of the control men walked for at least 4 hrs/week At the initial checkup 97 % *of both the female groups were sedentary*

All the other groups except that of control women *increased their leisure time activity during the* follow up year After the year 25 6 % of the training men 38.8 % of the control men, 57 1 % of the training women and 79 3 of the control women were sedentary

Both the training men and the control men were significantly more active physically at the time of the 3-month checkup than they had been prior to their infarction ($p < 0.01$) and the physical activity of both groups continued to increase until the end of the year Although the training men were somewhat more active physically *than the* controls, no statistically significant differences in *leisure time physical activity appeared* between the groups during the follow up year Neither was *there any difference* in leisure time physical activity between the training women and the control women at any checkup The training women were however more active than the controls, and clearly increased their physical activity compared with the pre infarctional time ($p < 0.05$)

$p < 0.01$). The initial checkup revealed no differences between the training men and the control men in pulmonary congestion. The control men had more pulmonary congestion after 9 and 12 months, but the differences were not, however significant.

The training women had almost significantly more left ventricular hypertrophy at the initial checkup than the controls (51.4 % and 23.5 % $p < 0.05$). In the subsequent checkups the training women continued to display more LVH but the differences were not significant. The training women also had more left atrial hypertrophy (LAH) at all the checkups than the control women, but only after 9 months was the difference almost significant (29.0 % and 6.9 % $p < 0.05$)

Metabolic variables

(Tables XL and XLI)

The mean serum cholesterol of the control men was almost significantly higher after 9 months than after 3 months (291 mg/100 ml and 284 mg/100 ml, $p < 0.05$). In the other patient groups, the mean serum cholesterol value did not change significantly during the follow-up period. Throughout the follow-up period, the control men had slightly higher mean serum cholesterol value than the training men, and the control women slightly higher value than the training women. The differences were not, however significant at any stage.

There was no change in the mean serum triglycerides within any group during the year and no significant differences were noted between the training and the control groups.

In addition, no significant differences between the training men and control men or the training women and the control women recorded in the mean 2 hour glucose tolerance. The training men had an almost significantly higher value in the 2 hour glucose tolerance test after 12 months than after 3 months (105 mg/100 ml and 98 mg/100 ml, $p < 0.05$)

The mean serum urate content after 6 months was 5.97 mg/100 ml in the group of control women and 4.76 mg/100 ml in the group of training women, the difference being almost significant ($p < 0.05$). Not other checkup revealed any differences between the training men and the control men or the training women and the control women. The mean serum urate of the control men was almost significantly lower after 6 months than after 3 months (5.51 mg/100 ml and 5.94 mg/100 ml, $p < 0.05$). No other group displayed any significant change of the serum urate content during the follow-up period.

Ergometric tests

Reason for the interruption of pedalling
(Table XLII)

Among the training men, control men, training women and control women who pedalled until their subjective maximum, no significant differences were noted on any occasion between those who interrupted the ergometric test for different reasons. Among the training men, however the relative proportion of those interrupting because of chest pain continually increased: in the first test 43.1 % discontinued because of chest pain, while in the 12-month test 73 % did so the difference being highly significant ($p < 0.001$). The proportion retiring because of chest pain did not change significantly during the follow-up in the other groups.

Physical working capacity 130 (Tables XLIII, XLIV XLV XLVI and XLVII Figures 1 and 2)

The physical working capacity 130 (PWC 130), in the first ergometric test, was on average 465.9 kpm/min in the group of training men, and on average 526.2 kpm/min in the group of control men. The difference was significant ($p < 0.01$). The mean PWC 130 of both the training men and the control men was highly significantly greater than initially from the 3-month ergometric test onwards (Table XLIII) The mean PWC 130 of the training men no longer increased after the 3-month test, nor did the PWC 130 of the control men increase after the 6-month test. At the end of the follow-up year the training men had a mean PWC 130 of 533.6 kpm/min, and the control men 619.1 kpm/min. The difference was significant ($p < 0.01$). The mean PWC 130 of the training men had increased by 14.5 % when compared with the initial test, while that of the control men increased by 17.7 %

By the end of the follow-up year the PWC 130 had increased by more than 10 % in 46 (52.9 %) training and 64 (58.7 %) control men (Table XLVI). The difference was not significant. The PWC 130 value had increased by more than 20 % in 34 (39.1 %) training men and 48 (44.0 %)

months and highly significantly lower at 12 months than initially The mean systolic blood pressure of the control men was almost significantly lower at 3 6 and 9 months than initially but at 12 months the value had returned to the initial level The mean systolic blood pressure of the training women was almost significantly lower at 12 months than initially while that of the control women was almost significantly lower at 3 months than initially (Table XXXIV)

Systolic blood pressure increased in 41 (33 6 %) training men and 55 (39 6 %) control men and declined in 68 (55 8 %) training men and 70 (50 4 %) control men The differences were not significant.

Systolic blood pressure increased in 8 (26.7 %) training women and 14 (48.3 %) control women during the follow up year and declined in 21 (70 %) training women and 12 (41 4 %) control women. The difference was almost significant ($p < 0.05$)

None of the checkups revealed any significant differences in the mean diastolic blood pressure between the training men and the control men, or the training women and the control women (Table XXXV) The mean diastolic blood pressure of the training men was significantly ($p < 0.01$) lower at the 6-month checkup and almost significantly ($p < 0.05$) lower after 12 months. The mean diastolic blood pressure of the training women was almost significantly ($p < 0.05$) lower after 12 months. Both the control men and the control women had a significantly lower mean diastolic blood pressure after 3 months but the values recorded at the subsequent checkups did not differ from the initial value (Table XXXV)

During the follow up year the diastolic blood pressure increased in 42 (34 4 %) training men and 5? (38 %) control men and declined in 53 (43.5 %) training men and 57 (41 6 %) control men. The differences were not significant. During the same year the diastolic blood pressures of 4 (13 3 %) training women and 14 (48.3 %) control women were elevated, and the pressures of 15 (50 %) training women and 11 (38 %) control women declined. The difference was almost significant ($p < 0.05$)

ECG findings

(Tables XXXVI and XXXVII)

None of the checkups showed any differences between the training men and the control men, or the training women and the control women in the frequency of QS changes or ST changes, aneurysms, AV blocks, branch blocks or the occurrence of extrasystoles.

Although there was no significant difference at any stage between the training men and the control men in the frequency of ST changes, the training men had almost significantly ($p < 0.05$) less ST changes at the 3-month and 6-month checkups, and significantly ($p < 0.01$) less ST changes after 9 and 12 months. No significant change took place in the frequency of ST changes in the group of control men during the follow-up year

Roentgenologic findings

(Tables XXXVIII and XXXIX)

The mean relative heart volume at the initial checkup was 495 8 cm /m in the training men and 492.3 cm /m in the control men (the difference was not significant) and 487 0 cm /m in the training women and 451 2 cm /m in the control women (the difference was not significant).

During the follow up period the mean relative heart volume of both the training men and the training women increased. The volume recorded for the training men at the 9-month and 12 month checkups was almost significantly greater ($p < 0.05$) than initially while the value recorded for the training women at 6 months was significantly ($p < 0.01$) greater that at 9 months being highly significantly ($p < 0.001$) greater and that at 12 months almost significantly ($p < 0.05$) greater than initially (Table XXXVIII) The mean relative heart volume of both the control men and the control women declined during the follow up year The changes were not significant in either group

The mean relative heart volume of the training men was almost significantly ($p < 0.05$) greater than that of the control men from the 6-month checkup onwards, and the mean relative heart volume of the training women was, similarly almost significantly ($p < 0.05$) greater than that of the control women from the 9-month checkup onwards. At the 12 month checkup the mean relative heart volume of the training men was 505 5 cm m that of the control men 473 9 cm m that of the training women 529.8 cm /m and that of the control women 441 6 cm /m

At every checkup the training men had more left ventricular hypertrophy (LVH) than the control men (Table XXXIX) but the difference was only significant after 9 months (47 2 % and 30.5 %

$p < 0.01$) The initial checkup revealed no differences between the training men and the control men in pulmonary congestion. The control men had more pulmonary congestion after 9 and 12 months, but the differences were not, however significant.

The training women had almost significantly more left ventricular hypertrophy at the initial checkup than the controls (51.4 % and 23.5 % $p < 0.05$). In the subsequent checkups the training women continued to display more LVH, but the differences were not significant. The training women also had more left atrial hypertrophy (LAH) at all the checkups than the control women, but only after 9 months was the difference almost significant (29.0 % and 6.9 % $p < 0.05$)

Metabolic variables

(Tables XL and XLI)

The mean serum cholesterol of the control men was almost significantly higher after 9 months than after 3 months (291 mg/100 ml and 284 mg/100 ml, $p < 0.05$). In the other patient groups, the mean serum cholesterol value did not change significantly during the follow-up period. Throughout the follow-up period, the control men had a slightly higher mean serum cholesterol value than the training men, and the control women a slightly higher value than the training women. The differences were not, however significant at any stage.

There was no change in the mean serum triglycerides ithin any group during the year and no significant differences ere noted between the training and the control groups.

In addition, no significant differences between the training men and control men or the training women and the control women recorded in the mean 2 hour glucose tolerance. The training men had an almost significantly higher value in the 2-hour glucose tolerance test after 12 months than after 3 months (105 mg/100 ml and 98 mg/100 ml, $p < 0.05$).

The mean serum urate content after 6 months as 5.97 mg/100 ml in the group of control women and 4.76 mg/100 ml in the group of training women, the difference being almost significant ($p < 0.05$) Not other checkup revealed any differences between the training men and the control men or the training women and the control women. The mean serum urate of the control men was almost significantly lower after 6 months than after 3 months (5.51 mg/100 ml and 5.94 mg/100 ml, $p < 0.05$) No other group displayed any significant change of the serum urate content during the follow-up period.

Ergometric tests

Reason for the interruption of pedalling (Table XLII)

Among the training men control men, training women and control women who pedalled until their subjective maximum, no significant differences were noted on any occasion between those who interrupted the ergometric test for different reasons. Among the training men however the relative proportion of those interrupting because of chest pain continually increased. In the first test 43.1 % discontinued because of chest pain, while in the 12 month test 73 % did so the differences being highly significant ($p < 0.001$). The proportion retiring because of chest pain did not change significantly during the follow-up in the other groups.

Physical working capacity 130: (Tables XLIII XLIV XLV XLVI and XLVII Figures 1 and 2)

The physical working capacity 130 (PWC 130) in the first ergometric test, was on average 465.9 kpm/min in the group of training men, and on average 526.2 kpm/min in the group of control men. The difference was significant ($p < 0.01$). The mean PWC 130 of both the training men and the control men was highly significantly greater than initially from the 3-month ergometric test onwards (Table XLIII) The mean PWC 130 of the training men no longer increased after the 3-month test, nor did the PWC 130 of the control men increase after the 6-month test. At the end of the follow-up year the training men had a mean PWC 130 of 533.6 kpm/min, and the contral men 619.1 kpm/min The difference was significant ($p < 0.01$). The mean PWC 130 of the training men had increased by 14.5 % when compared with the initial test, while that of the control men increased by 17.7 %

By the end of the follow-up year the PWC 130 had increased by more than 10 % in 46 (52.9 %) training and 64 (58. %) control men (Table XLVI). The differences was not significant. The PWC 130 value had increased by more than 20 % in 34 (39.1 %) training men and 48 (44.0 %)

months and highly significantly lower at 12 months than initially The mean systolic blood pressure of the control men was almost *significantly lower at* 3 6 and 9 months than initially but at 12 months the *value had returned to the initial level* The mean systolic blood pressure of the training women was almost significantly lower at 12 months than initially while that of the control women was almost significantly lower at 3 months than initially (Table XXXIV)

Systolic blood pressure increased in 41 (33 6 %) training men and 55 (39 6 %) control men, and declined in 68 *(55 8 %) training men and 70 (50 4 %)* control men *The differences were not signifi cant*

Systolic blood pressure increased in 8 (26.7 %) training women and 14 (48.3 %) control women during the follow up year and declined in 21 (70 %) training women and 12 (41 4 %) control women The difference was almost significant ($p < 0\ 05$)

None of the checkups revealed any significant differences in the mean diastolic blood pressure between the training men and the control men or the training women and the control women (Table XXXV) The *mean diastolic blood pressure of the* training men was significantly ($p < 0\ 01$) lower at the 6-month *checkup and almost significantly* ($p < 0\ 05$) lower after 12 months. The mean di astolic blood pressure of the training women was almost significantly ($p < 0\ 05$) lower after 12 months. Both the control men and the control women had a significantly lower mean diastolic blood pressure after 3 months, but the values recorded at the subsequent checkups did not differ from the initial value (Table XXXV)

During the follow-up year the diastolic blood pressure increased in 42 (34 4 %) training men and 52 (38 %) control men, and declined in 53 (43.5 %) training men and 57 (41 6 %) control men. The differences were not significant During the same year the diastolic blood pressures of 4 *(13.3 %) training women* and 14 (48.3 %) control women were elevated and the pressures of 15 *(50 %) training women and 11 (38 %)* control women declined. The difference was almost significant *($p < 0\ 05$)*

ECG findings

(Tables XXXVI and XXXVII)

None of the *checkups* showed any differences between the training men and the control men or the training *women and the* control women in the *frequency of QS* changes or *ST* changes, aneurysms, AV blocks branch blocks or the occurrence of extrasystoles.

Although there was no significant difference at any stage between the training men and the control men in the frequency of *ST* changes, the training men had almost significantly ($p < 0\ 05$) less *ST* changes at the 3-month and 6-month checkups, and significantly ($p < 0\ 01$) less *ST* changes after 9 and 12 months. *No significant change took place* in the frequency of *ST* changes in the group of *control men during the follow-up year*

Roentgenologic findings

(Tables *XXXVIII* and *XXXIX*)

The mean relative heart volume at the initial checkup was 495 8 cm /m² in the training men and 492.3 cm /m in the control men (the difference was not significant) and 487 0 cm /m in the training women and 451.2 cm /m in the control women (the difference was not significant)

During the follow up period the mean relative heart volume of both the training men and the *training women increased. The volume recorded for* the training men at the 9-month and 12 month checkups was almost *significantly greater ($p < 0.05$)* than initially while the value recorded for the training women at 6 months was *significantly* ($p < 0\ 01$) greater that at 9 months being highly significantly ($p < 0\ 001$) greater and *that at 12* months almost significantly ($p < 0\ 05$) greater than initially (Table XXXVIII) The mean relative heart volume of both the control men and the control women declined *during the follow up year* The changes were not significant in either group

The mean relative heart volume of the training men was almost significantly ($p < 0\ 05$) greater than that of the control men from the 6-month checkup onwards, and the mean relative heart volume of the training women was, similarly almost significantly ($p < 0\ 05$) greater than that of the control women from the 9-month checkup onwards. At the 12 month checkup the mean relative heart volume of the training men was 505.5 cm /m that of the control men 473 9 cm m that of the training women 529 8 cm /m and that of the control women 441 6 cm /m

At every checkup the training men had more left ventricular hypertrophy (LVH) than the control men (Table XXXIX) but the *difference* was only significant after 9 months (47 2 % and 30.5 %

where it was on average 3578 kpm, i.e. 40.7 % higher than in the first test. At the end of the follow-up year the mean SM of the training men was 3548 kpm, i.e. 39.3 % higher than in the initial test. The 9-month value was significantly higher ($p < 0.01$) and the 12 month value almost significantly higher ($p < 0.05$) than the initial value. The SM of the control men increased less than that of the training men. The SM of the control men as highest in the 6-month test, being on average 2706 kpm, i.e. 38.5 % higher than in the first test. The increase was not significant. At the end of the follow up the SM of the control men was on average 2573 kpm, i.e. 31 7 % higher than in the first test. The difference was not significant. In the 9-month test, the mean SM of the training men was almost significantly higher than that of the control men ($p < 0$ 05) At the other testing times the difference between the training men and the control men was not significant.

The mean subjective maximum of the training omen and control women did not change significantly during the follow-up year 1 the first ergometric test, the SM of the training women was on average 1748 kpm, while that of the control omen was 1228 kpm. The difference was not significant. The SM of the training women was highest in the 3-month test, here it was on average 2026 kpm, i.e. 15 9 % higher than in the first test. At the end of the year the mean SM of the training women was 1598 kpm or 8.6 % lower than in the initial test The SM of the control omen was highest in the 9-month test, hen the mean was 1406 kpm, or 14 7 % higher than initially At the end of the follow-up year the mean SM of the control women was 1350 kpm, or 9 9 % higher than in the first test In the 3-month test, the mean SM of the training omen was almost significantly higher than that of the control women ($p < 0$ 05) No other test revealed any differences between the training women and the control omen (Table XLVIII Figure 3).

When w examine the combined male-female groups, w see that the subjective maximum of the training subjects in the first test as on average 2459 kpm, hile that of the controls 1771 kpm. The difference as almost significant ($p < 0.05$). The SM of the trainers was highest in the 9-month test, here the mean as 3312 kpm, or 34 7 % higher than initially At the end of the follow-up year the mean SM of the trainers as 3252 kpm, 32 2 % higher than initially The mean SM of the training subjects was significantly ($p < 0.01$) higher after 3 and 9 months, and almost significantly higher ($p < 0$ 05) after 6 and 12 months than in the one-month test.

The subjective maximum of the controls was highest at the end of the follow up year when the mean was 2290 kpm, or 29.3 % higher than in the first test. The mean SM of the controls was almost significantly higher ($p < 0.05$) after 3 and 6 months than in the one-month test.

The mean subjective maximum of the training subjects was significantly higher ($p < 0.01$) after 3 and 9 months, and almost significantly ($p < 0.05$) higher after 6 months than the SM of the controls. The difference at the end of the follow-up year was not significant (Table XLVIII, Figure 3)

Pulse rate and blood pressure (Tables XLIX, L and LI)

The mean pulse rate, recorded before the ergometric test, was always highly significantly higher ($p < 0.001$) in the male training group than in the group of control men from the 3-month test onwards. The training subjects had already done some callistenic before taking the ergometer and although a few minutes were allowed for recovery prior to the test, the pulse rate apparently did not return to the basal level. The means of the initial pulse rates of the training men and control men were hence, probably not comparable. The mean pulse rate recorded at the end of the test, was also always significantly ($p < 0.01$) higher in the group of training men than among the controls from the 3-month test onwards (Table XLIX). The mean initial pulse of the training women was almost significantly higher ($p < 0.05$) after 3 and 6 months, and highly significantly ($p < 0.001$) higher after 9 and 12 months than the corresponding value of the controls, but for the same reason as in the male group the initial pulses of the female groups were apparently not comparable. The mean final pulse of the training women was almost significantly higher ($p < 0.05$) than that of the control women, after 3 and 9 months (Table XLIX).

None of the results revealed any differences in the mean systolic blood pressure recorded prior to the ergometric test, between the training men and the control men or the training women and the control women (Table L). The mean systolic blood pressure, *recorded at the end of the ergometric* test, as significantly higher ($p < 0.01$) among the control men than among the training men after 12 months. Otherwise there were no significant differences in the mean end-systolic pressures between the training groups and the control groups.

The mean diastolic blood pressure, recorded before the ergometric test, was on all occasions slightly higher in the group of training men than

control men (Table XLVII) The difference was not significant

Those training men and control men who pedalled until their subjective maximum in the tests increased their PWC 130 on average more than the others, and they continued to increase it until the last test (Table XLIII) These training men had a mean PWC 130 of 562.6 kpm/min at the end of the year and these control men reached a mean value of 716 0 kpm/min. The difference was almost significant ($p < 0.05$) The increase recorded for the training men was 22.0 % and that for the control men 32.8 %

The PWC 130 of the training women was on average 328 4 kpm/min in the first ergometric test, while that of the control women was on average 323.5 kpm/min In both of the female groups the mean PWC 130 values increased significantly during the follow up year (Table XLIV) The mean PWC 130 of the training women was at its highest at the 6-month ergometric test when it was 405 6 kpm/min, i.e 23.5 % higher than in the initial test ($p < 0.01$) Thereafter however the mean PWC 130 values of the training women declined, so that the mean PWC 130 after 12 months was 358 7 kpm/min i.e 9 2 % higher than initially In the group of control women on the other hand the mean PWC 130 continued to increase till the end of the follow up year In the last test the mean PWC 130 of the control women was 411 4 kpm/min, which is higher by 27 2 % than the initial value ($p < 0.001$) However at no stage was the difference between the training women and the control women significant.

PWC 130 improved by more than 10 % in 10 (43.5 %) training women and 12 (57 2 %) control women The difference was not significant. PWC 130 improved by more than 20 % in 9 (39 1 %) training women and 10 (47 6 %) control women The difference was not significant. (Tables XLVI and XLVII)

Those women both training women and controls, who pedalled until their subjective maximum in the tests also increased their mean PWC 130 more than others (Table XLIV) At the end of the year the PWC 130 of these training women was on an average 385 0 kpm/min i.e 17 0 % higher than initially while the value recorded for these control women was on an average 500 8 kpm/min i e 48 0 % higher than initially The difference between the training women and the control women was not significant

When the men and women were combined to make one control group and one training group the mean PWC 130 of the training subjects in the initial ergometric test was 438 6 kpm min and that of the controls 495 0 kpm/min The difference was significant ($p < 0.01$) From the 3-month test onwards both groups consistently displayed highly significantly higher ($p < 0.001$) mean PWC 130 values than initially (Table XLV) In the training group the mean PWC 130 no longer increased after the 3-month test and in the control group no essential changes took place after the 6-month test. At the end of the follow up year the mean PWC 130 of the training group was 497 0 kpm/min, i.e 13 3 % higher than initially and the mean 12 month value of the control group was 585 6 kpm/min i e. 18.3 % higher than initially The difference was highly significant ($p < 0.001$)

By the end of the follow up year the PWC 130 value of 56 (50 9 %) training subjects and 76 (58.5 %) controls had improved by more than 10 % The difference was not significant (Table XLVI) The PWC 130 of 43 (39 1 %) training subjects and 58 (44 6 %) controls had improved by more than 20 % by the end of the year The difference was not significant (Table XLVII) The proportion of those with an improvement of over 10 % did not increase after the 3-month test in the training group or after the 6-month test in the control group The proportion of those with an improvement of over 20 % did not increase in either group after the 6th month

The mean PWC 130 values and the numbers of the subjects, with an improvement of more than 10 % in PWC 130 show that the trainers reached the upper limit of the improvement of their physical working capacity within 3 months while the controls only reached this level after 6 months

The PWC 130 of the training subjects able to pedal until their subjective maximum was on an average 533.0 kpm/min at the end of the year i.e 19 7 % higher than in the first test The PWC 130 of the controls who pedalled until their subjective maximum was on average 654 5 kpm min at the end of the year which is 34 6 % more than in the initial test The increase in the training group was significant ($p < 0.01$) and that in the control group highly significant ($p < 0.001$) while the difference between the two groups was almost significant ($p < 0.05$) (Table XLV)

Subjective maximum (Table XLVIII Figure 3)

The subjective maximum of the training men in the first ergometric test was on average 2544 kpm while that of the control men on average 1951 kpm The difference was not significant The SM of training men was highest in the 9 month test

where it was on average 3578 kpm, i.e. 40.7 % higher than in the first test. At the end of the follow-up year the mean SM of the training men as 3548 kpm, i.e. 39.3 % higher than in the initial test. The 9-month value was significantly higher ($p < 0.01$) and the 12 month value almost significantly higher ($p < 0.05$) than the initial value. The SM of the control men increased less than that of the training men. The SM of the control men as highest in the 6-month test, being on average 2705 kpm, i.e. 38.5 % higher than in the first test. The increase was not significant. At the end of the follow-up the SM of the control men was on average 2573 kpm, i.e. 31.7 % higher than in the first test. The difference was not significant. In the 9-month test, the mean SM of the training men was almost significantly higher than that of the control men ($p < 0.05$). At the other testing times the difference between the training men and the control men was not significant.

The mean subjective maximum of the training women and control women did not change significantly during the follow-up year. In the first ergometric test, the SM of the training women was on average 1748 kpm, while that of the control women was 1228 kpm. The difference was not significant. The SM of the training women was highest in the 3-month test, where it was on average 2026 kpm, i.e. 15.9 % higher than in the first test. At the end of the year the mean SM of the training women was 1598 kpm, or 8.6 % lower than in the initial test. The SM of the control omen was highest in the 9-month test, when the mean 1408 kpm, or 14.7 % higher than initially. At the end of the follow-up year the mean SM of the control women as 1350 kpm, or 9.9 % higher than in the first test. In the 3-month test, the mean SM of the training women was almost significantly higher than that of the control women ($p < 0.05$). No other test revealed any differences between the training women and the control omen (Table XLVIII Figure 3).

When examine the combined male-female groups see that the subjective maximum of the training subjects in the first test was on average 2459 kpm, while that of the controls 1771 kpm. The difference as almost significant ($p < 0.05$). The SM of the trainers was highest in the 9-month test, here the mean was 3312 kpm, or 34.7 % higher than initially. At the end of the follow-up year the mean SM of the trainers was 3252 kpm, i.e. 32.2 % higher than initially. *The mean SM* of the training subjects was significantly ($p < 0.01$) higher after 3 and 9 months, and almost significantly higher ($p < 0.05$) after 6 and 12 months than in the one month test.

The subjective maximum of the controls was highest at the end of the follow-up year when the mean was 2290 kpm, or 29.3 % higher than in the first test. The mean SM of the controls was almost significantly higher ($p < 0.05$) after 3 and 6 months than in the one month test.

The mean subjective maximum of the training subjects was significantly higher ($p < 0.01$) after 3 and 9 months, and almost significantly ($p < 0.05$) higher after 6 months than the SM of the controls. The difference at the end of the follow-up year was not significant (Table XLVIII Figure 3)

Pulse rate and blood pressure
(Tables XLIX, L and LI)

The mean pulse rate, recorded before the ergometric test, was always highly significantly higher ($p < 0.001$) in the male training group than in the group of control men from the 3-month test onwards. The training subjects had already done some challstenic before taking the ergometer and although a few minutes were allowed for recovery prior to the test, the pulse rate apparently did not return to the basal level. The means of the initial pulse rates of the training men and control men were, hence, probably not comparable. The mean pulse rate, recorded at the end of the test, was also always significantly ($p < 0.01$) higher in the group of training men than among the controls from the 3-month test onwards (Table XLIX). The mean initial pulse of the training women was almost significantly higher ($p < 0.05$) after 3 and 6 months, and highly significantly ($p < 0.001$) higher after 9 and 12 months than the corresponding value of the controls, but for the same reason as in the male group, the initial pulses of the female groups were apparently not comparable. The mean final pulse of the training women was almost significantly higher ($p < 0.05$) than that of the control women, after 3 and 9 months (Table XLIX).

None *of the results revealed* any differences in the mean systolic blood pressure recorded prior to the ergometric test, between the training men and the control men or the training women and the control women (Table L). *The mean* systolic blood pressure, recorded at the end of the ergometric test, as significantly higher ($p < 0.01$) among the control men than among the training men after 12 months. Otherwise there were no significant differences in the mean end-systolic pressures between the training groups and the control groups.

The mean diastolic blood pressure recorded before the ergometric test, was on all occasions slightly higher in the group of training men than

among the control men, but the differences were not significant. The mean diastolic blood pressure, obtained at the end of the ergometric test, was highly significantly higher ($p < 0.001$) after 1 and 3 months and almost significantly higher ($p < 0.05$) after 6 months, in the group of training men than among the control men (Table LI)

There were no significant differences between the training women and the control women in the mean diastolic blood pressure measured before and after the ergometric test (Table LI)

Pulse rate — blood pressure product: (Table LII Figure 4)

The mean pulse rate — blood *pressure product*, obtained at the end of the ergometric test increased slightly in both the group of training men and the group of control men The mean pulse rate — blood pressure product of the training men was 22.9×10^3 in the first test while that of the control men was 22.4×10^3 The mean pulse rate — blood pressure product of the training men was highest after 9 months, 24.4×10^3 (almost significantly higher than the mean of the first month $p < 0.05$) and that of the control men after 12 months: 23.6×10^3 (almost significantly higher than the first month s mean $p < 0.05$) After 9 months the mean pulse rate — blood pressure product of the training men was almost significantly higher than that of the control men ($p < 0.05$) In the other tests, the training men and the control men did not differ significantly with regard to the pulse rate — blood pressure product.

The mean pulse rate — *blood pressure product* of the control women did not exceed the first month s mean in any of the tests, and in the group of training women only the 3-month test value was higher than the initial value. In the 3-month test the mean pulse rate — blood pressure product of the training women was 27.5×10^3 and the mean of the control women 23.9×10^3 The difference was significant ($p < 0.01$) (Table LII)

Return to work

At the onset of the infarction 95 (66 4 %) of the training men, 109 (65 7 %) *of the control men* 27 (73 %) of the training women and 15 (44 1 %) of the control women were able to work (Table VII)

The number of those *on sickness pension* was 39 (27.3 %) in the group of training men, 45 (27 1 %) among the control men 9 (24.3 %) among the training women and 14 (41.2 %) among the control women. Eight (5.6 %) of the training men 11 (6 6 %) of the control men, 1 (2.7 %) of the training women and 3 (8.8 %) of the control women were on sick leave (Table VII)

The cause of retirement was a previous infarction in the case of 17 (36 2 %) training men 20 (35.7 %) control men 4 (40 %) training women and 7 (41.2 %) control women (Table VIII)

Among those not capable of work, the cause of retirement had been a coronary disease without infarction in the case of 18 (38 3 %) training men, 23 (41 1 %) control men, 5 (50 %) training women and 8 (47 1 %) control women Coronary disease was the reason for the disability of 35 (74.5 %) training men on pension or sick leave 43 (76 8 %) control men 9 (90 %) training women and 15 (88 2 %) control women (Table VIII)

Of those training men who were able to work at the onset of infarction 45 (47 4 %) returned to work after sick leave Five patients were subsequently obliged to retire because of abundant symptoms. Hence 40 (42.1 %) men finally recovered their working capacity and 3 of them took a less *strenuous job than previously Thus, altogether* 37 (39 %) training men resumed their previous employment (Table LIII)

Of the control men able to work at the onset of infarction, 39 (39 4 %) resumed their previous employment after the sick leave Three patients had later to retire. Altogether 36 (33 %) ultimately recovered their working capacity One man moved into a less strenuous occupation 35 (32.1 %) *control men returned to their previous work permanently There was no significant difference* between the training men and the control men in the resumption of work.

In the *female groups* 7 (25 9 %) training women and 5 (33.3 %) control women returned permanently to their *previous employment In addition one training woman and 2 control women were able to resume their old job for a short while None of the* women moved into a less strenuous occupation (Table LIII) *There was no significant difference* between the training women and the control women in the resumption of work

In all the patient groups the subjects who recovered their working capacity returned to work within a *year from the onset of infarction None were on sick lea e for more* than 12 month More than half of those who ultimately res med work in each patient group were working 6 months after the onset of infarction (Table LIV)

Prognosis

The training group included 143 men and 37 women, altogether 180 patients, who were followed up for an average of 31 1/2 months. The control group consisted of 166 men and 34 women, altogether 200 patients, all of whom were followed up for an a erage of 26 1/2 months.

During the follow up period, 21 (11 7 %) training patients experienced a total of 27 recidivous infarctions. Fifteen (10.5 %) of the training men had one recidivous infarction, 1 (0 7 %) had two, and 2 (1 4 %) had three. Altogether 18 (12.6 %) training men had re-infarction. Two (5 4 %) training women had one recidivous infarction and 1 (2.7 %) had two. Altogether 3 (8.1 %) training women had a re-infarction (Table LV and Figure 5)

In the control group 29 (14.5 %) patients had a total of 33 recidivous infarctions. Twenty two (13.3 %) of the control men had one recidivous infarction and 1 (0.6 %) had two. Altogether 23 (13.9 %) control men had a re-infarction Three (8.8 %) control women had one recidivous infarction and 3 (8 8 %) had two. Altogether 6 (1 .6 %) control women had re-infarction (Table LV and Figure 5). There was no difference in recidivation between the training group and the control group

The average time hich elapsed before the first recidivous infarction was 12.6 months in the group of training men and 10.4 months in the group of control men. The difference was not significant. In the group of training women the average time hich elapsed before the first recidivous infarction as 12.0 months, hile the corresponding time in the group of control women as 8 3 months. The difference as not significant (Table LVI)

During the follow up period, 19 (10 6 %) training subjects died, 17 (11 9 %) men and 2 (5 4 %) omen. In the control group 33 (16.5 %) subjects died, 27 (16.3 %) men and 6 (17 7 %) women (Table LVII, Figure 6) The differences between the training subjects and the controls were not significant.

The average time lapse between the infarction and the death was 19 4 months in the group of training men and 11 8 months in the group of control men. The difference was almost significant ($p < 0.05$) The time between the infarction and the death was on average 12.0 months among the training women and 11.0 months among the control women. The difference was not significant (Table LVII)

In the group of training men, 16 out of 17 died of a coronary disease, 1 died 13 months after the infarction of lung cancer Among the control men 22 out of 27 died a coronary death; reasons for the five other deaths were as follows. cerebral hemorrhage 1 month after the infarction, accident 3 months after the infarction, ventricular cancer 12 months after the infarction, suicide 31 months after the infarction, and sepsis 32 months after the infarction. All those women who died succumbed to a coronary disease (Table LVIII).

Coronary deaths numbered 18 (10 %) in the training group and 28 (14 %) in the control group The difference was not significant.

A sudden death was suffered by 10 training subjects (9 men and 1 women) i.e. 55 6 % of those with coronary death, and 15 controls (13 men and 2 women), i.e 53 7 % of those with a coronary death (Table LVIII). Most of the coronary deaths in all the patient groups took place at rest. One training man died during a training session. Altogether trainers (6 men and 1 women) died during physical activity i.e. 39 % of the CHD deaths, the corresponding figure for the controls being 11 (10 men and 1 woman) or 39.3 % of the CHD deaths. A majority of the deaths occurred in the daytime, hen the subject were awake.

Most of those who died were autopsied: 14 (82.4 %) of the training men, 18 (66.7 %) of the control men, one of the two training omen, and all the control women (Table LVIII).

Discussion

Material. The majority of earlier works dealing with the rehabilitation of myocardial infarction patients suffer from certain shortcomings: the reference material is either inadequate or entirely lacking (e g Hellerstein 1968, Gottheiner 1969 Rechnitzer et al 1972) or the number of patients is small owing to the demanding nature of the study (e.g Torkelson 1964 Uusitalo et al 1972) In addition to this it is likely that infarction patients who are more active or in better condition than the average have often been selected for rehabilitation studies, because the criterion in selecting patients for rehabilitation has been the subject s own interest (e g Gottheiner 1969) or the report of the attending physician (e.g Hellerstein 1968) Well controlled studies, covering sufficiently large series of infarction patients, have been presented by Kentala (1972) and Sanne (1973)

In the present study 180 training patients and 200 controls were collected as periodic groups No other series of infarction patients equally large and adequately controlled has been presented so far The patients were not selected All working-age (under 65 yrs) subjects were accepted into the series, with the exception of those with serious motor invalidity or severe psychic disease.

On the basis of the anamnestic data, the training men and the control men as well as the training women and the control women were mutually comparable The only significant difference between the training men and the control men was place of residence. 43 % of the training men and 60 % of the control men came from the country No significant differences were however noted in occupational distribution and the degree of the heaviness of work between the training men and the control men. Since the two groups did not differ as regards free time physical activity prior to infarction either the training men and the control men were mutually comparable with regard to physical activity despite the difference in place of residence

On the basis of the clinical findings and laboratory results of the initial checkup the training men and control men as well as the training women and control women were also comparable The training women had more left ventricular hypertrophy in the thorax roentgenogram than the control women but the two female groups did not differ as regards the relative heart volume.

Method. The objective in previous rehabilitation of patients with myocardial infarction has been conducted and supervised training, usually taking place in a hospital or a physiotherapeutic institute (Hellerstein 1969 Kentala 1972 Sanne 1973). The advantage of this practice is that the training can be performed in suitable degree and safety The numerous visits to the rehabilitation institute may however cause inconvenience, and the number of drop-outs has been great in studies covering fairly long periods (Kentala 1972, Sanne 1973). For some patients, repeated hospital visits are an unpleasant experience (Sanne 1971)

At the time when the present project was being launched there was hardly any information concerning the effect of programmed spontaneous "self training" at home on the improvement of physical condition and the prognosis after myocardial infarction Moreover since the distances in Northern Finland are great and the transport services poor the visit to a training session may take the patient a whole day which means that an ordinary" rehabilitation program requiring numerous visits to the training institute would hardly have been realizable without a great number of drop-outs. For these reasons, the author elected for a training program where the patients came to a physical department to learn a calisthenic program and performed the actual training at home Duplicated instructions were distributed to help them memorize the movement series Ergometric training was not possible at home

Participation in rehabilitation proved that a program of this kind is realizable after 6 months 78 % of the training men and 65 % of the training women were still participating and 67 % of the men and 65 % of the women after 12 months. Among those who continued to participate in training, 12 % of the men and 17 % of the women did insignificantly b t calisthenic exercises at home after 6 months, the corresponding figures after 12 months being 15 % of the men and 14 % of the women. Participation in rehabilitation was more enthusiastic than in the programs described

previously In Gottheiner's (1969) series, 60 % of the subjects participated until the end, but in Kentala's (1972) study only 33 % of the subjects showed 70 % participation, and in Sanne's (1973) series 52 % attended the training sessions after 6 months and only 40 % after a year. The patients apparently did not get tired of the monthly visits to the rehabilitation institute during one year; rather the visit made once a month perhaps enlivened their day-to-day existence. The obligation to make notes and the expectation of the next visit and test may have been, along with the hope of getting healthy, reasons for the good challenge activity at home.

The difficulty in controlled rehabilitation studies is that many of the control patients begin rehabilitation on their own initiative (cf. Kentala). In the present work, the control patients were told that their training consisted of the monthly ergometric test in the outpatient department and that no other training was necessary. Training was not, however, forbidden. No training control group was formed, for only 4 control men did significant training.

The training groups consisted of 5—7 subjects, and subjects of roughly the same age and similar condition were chosen, to ensure effective training in the teaching situations. The training was interval training which became progressively more strenuous every month. The objective in the calisthenic exercises was to render the subjects slightly breathless and sweating. ECG monitoring was not available in the group calisthenics or the ergometric tests. The patients' cardiac function was followed through auscultation during the ergometric test. A total of 2518 ergometric tests were made, the only complication was ventricular fibrillation experienced by one man, which was successfully defibrillated. The complication frequency agreed with the values reported in the literature. The combined morbidity-mortality frequency during exercise tests is appr. 4/10000 tests (Rochmis et al 1971). The lack of monitoring did not seem to have any effect on the number of complications. No complications occurred during the training sessions. One training man died suddenly soon after leaving the calisthenic session. No complications occurred during the calisthenics at home.

The effect of training was observed by determining the submaximal value of aerobic power measured as the work load at heart rate of 130 per minute (Physical Working Capacity 130). When calculating the reference value of aerobic power a pulse rate of 130 was chosen, because it was desirable to avoid excessive intra- or extrapolation, which would have been necessary in several cases, if PWC 150 had been used. Since the maximal AV oxygen difference is not significantly limited in coronary disease, the reduction of aerobic power in a coronary patient reflects the functional severity of the disease (Bruce et al 1973). The maximal oxygen intake capacity is lower by 25—40 % after myocardial infarction, than in a healthy population of the same age (Kasser and Bruce 1969, Bengtsson 1972). Using various indirect measurements and nomograms, it has been possible to indicate that training increases the maximal oxygen intake capacity after myocardial infarction by 20—30 %, i.e. brings it nearly to the level of healthy subjects of the same age (Hellerstein 1968, Clausen 1969, Detry 1971, Redwood 1972).

When the subjective maximum is used as a measure of rehabilitative improvement, the caution of the subject may affect the result of the first test. The patient is apprehensive after the infarction, and does not dare to strain himself maximally although the physician is present to encourage and to give a certain feeling of security. The subject may discontinue pedalling and report, as the reason, a complaint which would not actually require him to stop. In the present study, too, a sudden improvement in the results of those who pedalled until their subjective maximum was noted between the first and the second ergometric tests in all the other groups except the control women.

On the other hand, pedalling until one's subjective maximum is probably useful for the improvement of one's physical condition, as the subject realizes that he is capable of heavy strain, he feels encouraged to engage in more vigorous activity in his everyday life. It was probably because of this invisible training effect that in all the four patient groups the subjects, who pedalled until their subjective maximum, increased their mean physical working capacity 130 during the training period somewhat more than the others.

Subjective symptoms. After a training period, the infarction patient may endure greater physical strain before the appearance of angina pectoris than prior to training (Redwood et al 1972, Sanne 1973), and the symptoms of angina pectoris may diminish after the training period (Kellermann 1975). Placebo procedures may also diminish the symptoms of infarction patients (Zohman and Tobis 1967, Bergman and Varnauskas 1971). No reduction of angina pectoris symptoms need not, however, be necessarily associated with the improvement of the condition and physical working capacity of infarction patients, for the rehabilitation and activation of the patients may daily create more situations provoking pain than prior to rehabilitation. Kentala (1972), for example, was unable to demonstrate any reduction of chest pain

Discussion

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On the basis of the clinical findings and laboratory results of the initial checkup the training men and control men as well as the training women and control women were also comparable The training women had more left ventricular hypertrophy in the thorax roentgenogram than the control women, but the two female groups did not differ as regards the relative heart volume

Method. The objective in previous rehabilitation of patients with myocardial infarction has been conducted and supervised training usually taking place in a hospital or a physiotherapeutic institute (Hellerstein 1969 Kentala 1972, Sanne 1973) The advantage of this practice is that the training can be performed in suitable degree and safety The numerous visits to the rehabilitation institute may however cause inconvenience and the number of drop-outs has been great in studies covering fairly long periods (Kentala 1972, Sanne 1973) For some patients, repeated hospital visits are an unpleasant experience (Sanne 1971)

At the time when the present project was being launched there was hardly any information concerning the effect of programmed spontaneous "self training" at home on the improvement of physical condition and the prognosis after myocardial infarction. Moreover since the distances in Northern Finland are great and the transport services poor the visit to a training session may take the patient a whole day which means that an "ordinary" rehabilitation program requiring numerous visits to the training institute would hardly have been realizable without a great number of drop-outs. For these reasons, the author elected for a training program where the patients came to a physical department to learn a chalistenic program and performed the actual training at home. Duplicated instructions were distributed, to help them memorize the movement series. Ergometric training was not possible at home

Participation in rehabilitation proved that a program of this kind is realizable· after 6 months 78 % of the training men and 65 % of the training women were still participating and 67 % of the men and 65 % of the women after 12 months Among those who continued to participate in training 12 % of the men and 17 % of the women did insignificantly lit chalistenic exercises at home after 6 months, the corresponding figures after 12 months being 15 % of the men and 14 % of the women Participation in rehabilitation was more enthusiastic than in the programs described

previously. In Gottheiner's (1969) series, 60 % of the subjects participated until the end, but in Kentala's (1972) study only 33 % of the subjects showed 70 % participation, and in Sanne's (1973) series 52 % attended the training sessions after 6 months and only 40 % after a year. The patients apparently did not get tired of the monthly visits to the rehabilitation institute during one year: rather the visit made once a month perhaps enlivened their day-to-day existence. The obligation to make notes and the expectation of the next visit and test may have been, along with the hope of getting healthy, reasons for the good chalistenic activity at home.

The difficulty in controlled rehabilitation studies is that many of the control patients begin rehabilitation on their own initiative (cf. Kentala). In the present work, the control patients were told that their training consisted of the monthly ergometric test in the outpatient department and that no other training was necessary. Training was not, however, forbidden. No training control group was formed, for only 4 control men did significant training.

The training groups consisted of 5—7 subjects, and subjects of roughly the same age and similar condition were chosen, to ensure effective training in the teaching situations. The training was interval training which became progressively more strenuous every month. The objective in the chalistenic exercises was to render the subjects slightly breathless and sweating. ECG monitoring was not available in the group chalistenics or the ergometric tests. The patient's cardiac function was followed through auscultation during the ergometric test. A total of 2516 ergometric tests were made, the only complication was ventricular fibrillation experienced by one man, which was successfully defibrillated. The complication frequency agreed with the values reported in the literature. The combined morbidity-mortality frequency during exercise tests is appr. 4/10000 tests (Rochmis et al 1971). The lack of monitoring did not seem to have any effect on the number of complications. No complications occurred during the training sessions. One training man died suddenly soon after leaving the chalistenic session. No complications occurred during the chalistenics at home.

The effect of training was observed by determining the submaximal value of aerobic power measured as the work load at a heart rate of 130 per minute (Physical Working Capacity 130). When calculating the reference value of aerobic power a pulse rate of 130 was chosen, because it was desirable to avoid excessive intra- or extrapolation, which could have been necessary in several cases, if PWC 150 had been used. Since the maximal AV oxygen difference is not significantly limited in coronary disease, the reduction of aerobic power in a coronary patient reflects the functional severity of the disease (Bruce et al 1973). The maximal oxygen intake capacity is lower by 25—40 % after myocardial infarction than in a healthy population of the same age (Kasser and Bruce 1969, Benestad 1972). Using various indirect measurements and nomograms, it has been possible to indicate that training increases the maximal oxygen intake capacity after myocardial infarction by 20—30 %, i.e. brings it nearly to the level of healthy subjects of the same age (Hellerstein 1968, Clausen 1969, Detry 1971, Redwood 1972).

When the subjective maximum is used as a measure of rehabilitative improvement, the caution of the subject may affect the result of the first test. The patient is apprehensive after the infarction, and does not dare to strain himself maximally, although the physician is present to encourage and to give a certain feeling of security. The subject may discontinue pedalling and report, as the reason, a complaint which would not actually require him to stop. In the present study, too, a sudden improvement in the results of those who pedalled until their subjective maximum was noted between the first and the second ergometric tests in all the other groups except the control women.

On the other hand, pedalling until one's subjective maximum is probably useful for the improvement of one's physical condition, as the subject realizes that he is capable of heavy strain, he feels encouraged to engage in more vigorous activity in his everyday life. It was probably because of this invisible training effect that in all the four patient groups the subjects, who pedalled until their subjective maximum, increased their mean physical working capacity 130 during the training period somewhat more than the others.

Subjective symptoms. After a training period, the infarction patient may endure greater physical strain before the appearance of angina pectoris than prior to training (Redwood et al 1972, Sanne 1973), and the symptoms of angina pectoris may diminish after the training period (Kellerman 1975). Placebo procedures may also diminish the symptoms of infarction patients (Zohman and Tobis 1967, Bergman and Varnauskas 1971). No reduction of angina pectoris symptoms need not, however, be necessarily associated with the improvement of the condition and physical working capacity of infarction patients, for the rehabilitation and activation of the patients may daily create more situations provoking pain than prior to rehabilitation. Kentala (1972), for example, was unable to demonstrate any reduction of chest pain

or dyspnea during rehabilitation.

In the present work the frequency of dyspnea and chest pains increased in all the four patient groups during the follow up year This may have been due to the increased vigour and activity in everyday life. It was probably for the same reason that chest pains due to use of hands increased in all patient groups. The fact that all the patient groups contained a smaller percentage of subjects with daily chest pains at the end of the year than at the beginning may be indicative of a training effect and an improvement of physical condition Neither the training men and the control men nor the training women and the control women differed as regards the number of subjects with daily pains, which suggests that the improvement of physical working capacity was probably a natural phenomenon One indication of a possible favourable training effect was the difference noted in the prolonged chest pains of the training men and the control men, from the 3rd month onwards in the 3-month checkup and thereafter the control men had more prolonged chest pains, the difference being almost significant at the 9-month checkup

Leisure time physical activity The scarcity of leisure time physical activity among the patients prior to the onset of infarction attracts attention. Only 2 training men and 6 control men had engaged in physical activity with a significant training effect This physical inactivity agrees with findings which show lack of physical activity to be a risk factor in myocardial infarction (Fox and Haskell 1968, Sanne and Wilhelmsen 1970) It can also be thought that the lack of interest in physical activity might be a consequense, not a cause of the coronary disease It should be pointed out however that appr 20 % of the men and slightly less than 10 % of the women in this material were completely symptomless before the onset of infarction so that they could have been more active physically if they had so desired

The leisure time physical activity in the groups of training men and training women increased throughout the follow up year but the increase of activity in the control groups discontinued after 6 months From the 3-month checkup to the end of the year the training men were more active physically than the control men while the training women were more active than the control women from the 6-month checkup until the end of the year but the differences between the groups were not significant It appeared however that the training program encouraged the subjects to greater physical activity generally

The control patients did not begin to train on their own initiative in the present work as they did in Kentala s series. Only 4 control men started significant training during the follow up year

Fatigue, claudication In the group of training men fatigue decreased during the follow up period more than in the control group, with an almost significant difference by the 9-month checkup The difference may reflect the psychic effect of the training program Hellerstein and Hornsten (1968) demonstrated a tendency to depression and psychastenia after myocardial infarction Physical training served to improve the test values.

In the 3-month and 6-month checkups, the training men had significantly less claudication than the control men, and by 9 and 12 months the difference was almost significant. Since the training men and the control men displayed differences in claudication in the initial checkup (tr 13 % co 21 % the difference not significant) and since the claudication frequency of the training men did not decrease during the follow up the difference is hardly indicative of a training effect.

Smoking Training had no effect on smoking habits Neither the training men and the control men nor the training women and the control women differed as regards the number of smokers or the amount of tobacco consumed Kentala also found training to have no effect on smoking habits Pyörälä et al (1971) trained healthy-aged men who did not change their smoking habits, either Different results, however have also been presented. In Naughton s (1968) work 5 training subjects out of 14 and 6 controls out of 14 either cut down smoking or gave it up entirely Of Honegger s (1967) 80 infarction patients, as many as 77 % sopped smoking and 15 % cut it down

In the present work 39 (27 %) training men 48 (29 %) control men 7 (19 %) training women and 6 (18 %) control women gave up smoking after the infarction The corresponding figures in Kentala s study were of the same order Many of those who initially gave up smoking resumed it during the follow up year 12 training men 14 control men 2 training women and 4 control women did so. The smokers have a twofold mortality and morbidity after infarction compared with the non-smokers (Wilhelmsen et al 1975) Hence smoking, constitutes a significant risk factor even after the infarction. It is however difficult to see what is the cause and what is the effect The personality of the smoker may reflect a common cause for both mental stress and smoking and the infarction and smoking are not necessarily in a direct causal relationship Jenkins et al (1968) noted that Rosen man s personality type A (tense and easily stressed) included more subjects with ischemic heart disease and more smokers, than the more balanced type B

The smokers of both personality types, however had a clearly greater risk of developing coronary disease than the non-smokers.

Clinical findings. Pathologic S3 or ventricular gallop signifies diastolic overloading of the ventricle (Shah et al 1969). In myocardial damage S3 may be the first clinical sign of a collapse of cardiac power. Heikkilä et al (1971) noted that 80 % of 50 unselected infarction patients had S3 gallop at the acute stage, and in the cases where the infarction entailed cardiac collapse and resulted in death, S3 was always heard. In patients with angina pectoris, audible S3 can be provoked by strain (Mc Nair 1967). The ventricular gallop can be utilized in estimating the post-infarctional prognosis, for its appearance is a sign of poor prognosis (Heikkilä et al 1971).

The appearance of S4, or atrial gallop requires depressed compliance of the myocardium (Martinez-Lopez 1974). Pathologic atrial gallop is sign of intensified atrial contraction, which is a consequence of diminished ventricular compliance. The depressed compliance may be due to ventricular hypertrophy, ischemia of the wall and fibrosis. In the series of Heikkilä et al, S4 could be auscultated in 82 % of the infarction patients. S4 is so common in association with myocardial infarction that it has even been claimed that the diagnosis of infarction is uncertain without S4 (Turner et al 1973, Bethell 1973).

The persistence of S4 after infarction signifies a poor prognosis. Simultaneous persistence of both S3 and S4 is often associated with aneurysm (Heikkilä et al 1971). The fourth heart sound appears in the phonocardiogram of most middle-aged and old asymptomatic subjects (Spodick et al 1973, Swistak et al 1974). Juuttila (to be published) did not observe any difference in the frequency of S4 in the phonocardiogram, between healthy subjects and patients with angina pectoris, in a population sample from Northern Finland. The fourth heart sound heard in the auscultation of an asymptomatic subject may be a sign of pre-symptomatic ischemic heart disease (Turner et al 1973).

Pathologic fourth heart sound heard in auscultation is common and probably diagnostic in coronary disease, and it has prognostic significance after infarction.

During the follow up year the training and the control groups did not differ as regards the frequency of gallop sounds. The training women and the control women had an almost significant difference in S4 frequency in the initial checkup and a similar difference after 12 months.

If rehabilitation had had an adverse effect of manifesting latent states of cardiac decompensation, the S3 frequency could have been expected to increase in the training groups. This was not the case, however. If, on the other hand, rehabilitation had had favourable effects on cardiac hemodynamics, both the S3 and the S4 frequencies could have been expected to decrease. As the ischemia of the wall muscle and/or the nutrition would have been improved, the S4 frequency might have decreased and the S3 frequency would have been reduced by the improvement of decompensation. Yet no differences were noted in favour of the training subjects. It therefore appeared, that rehabilitation had no effect on cardiac hemodynamics when the gallop sounds were used as a scale.

In an unselected series of infarction patients, only part are able to train so intensively that a central training effect can be expected. Although there is indirect evidence showing that training may increase the coronary circulation of infarction patients, or improve the oxidation systems of the myocardium (Kattis and Frick 1970, Redwood et al 1972), it is also possible that a more important role in the training of coronary patients is played by the training of skeletal muscles, and the changes in peripheral circulation (Clausen et al 1969, Trap-Jensen 1970).

At the end of the follow-up year the control men and the control women had the same mean systolic and diastolic blood pressure values, as observed initially. The mean systolic and diastolic blood pressure values of the training men and the training women, on the other hand, were significantly lower at the end of the year. Although the differences between the training subjects and the controls were not significant, the decline of blood pressure in the training group may reflect a training effect. Some works on the training of infarction patients have revealed a significant decline of blood pressure (Naughton et al 1966, Hellerstein 1969) while some other studies have given no indication of decline: Pyfer and Doane found 3-month training to have no effect on blood pressure, and the one-year rehabilitation period in Kentala's (1972) work resulted in no change in blood pressure. It is also known that physically active subjects have less hypertension than inactive ones (Morris and Crawford 1960).

ECG changes. Training could not be shown to have any clear effect on the prevalence of QS and ST T changes. The training men displayed a significant decrease of ST T changes, but the difference in comparison with the controls was not significant. In the two female groups, both ST T changes and QS changes decreased during the follow-up year. The decreases were not significant,

and the groups did not *differ significantly from* each other

Kentala noted that the Q-QS changes of physically active patients disappear more rapidly than the corresponding changes of inactive ones The infor *mation given by resting ECG is apparently not* enough to show the possibly improved circulation, and more economical oxygen consumption of the myocardium. There is evidence showing that ischemic changes, in the exercise ECG of coronary *patients* are improved by training (Kattus et al 1968 Hellerstein et al 1969)

Roentgenologic findings. The initial differences in the mean relative heart volume, between the training men and the control men as well as the training women and the control women were not significant The mean relative heart volume values in the *male groups were of the same* order as those recorded in Kentala s work, and slightly higher than those obtained by Benestad (tr 496 cm /m co 492 cm /m³ Kentala 495/490 cm³/m *Benestad 463 cm /m*)

The *mean heart volume increased in the groups* of training men and training women throughout the follow up year It was almost significantly greater in both groups at the end of the year The mean relative heart volume of the control men and the control *women decreased slightly during the year* From the 6-month checkup onwards the training men had an almost significantly greater mean relative heart volume than the control men and the corresponding value of the training women was *almost significantly greater than that of the* control women from the 9 month checkup onwards What was the cause for the increase of heart volume in the training groups? Was it a development of insufficiency or a training effect on the myocardium.

The anamnestic data gave no suggestion that the training subjects would have had a greater tendency to heart failure than the controls, during the latter half of the follow up year From the 6-month checkup onwards the training groups displayed less congestion of the pulmonary veins as estimated from the thorax roentgenogram than the *control groups* which on the contrary seems to suggests that the training groups had a smaller tendency to cardiac insufficiency during the latter half of the follow up year

An increase of the heart size can be achieved through near maximal training in young people (Frick et al 1969) According to some authors, training does not increase the heart size in older people (*Saltin et al 1969*) but some others however have observed heart enlargement after training in elderly people (Mann 1969 Pyörälä et *al 1971*) *If the* increase in mean relative heart volume in the training groups were an indication of a central training effect, it could be expected that the reference values of aerobic power would also have been higher in the training groups than among the controls. This was not the case, however and the significance of the increase of heart size in the training *groups remains uncertain.*

Metabolism The mean cholesterol and triglyceride values did not decline in any patient group during the follow up year The lipid values were not different in the training and control groups. The *findings* were parallel to those of Varnauskas et al (1966) and Kentala (1972). After a training period, infarction patients have displayed a decline of both cholesterol (Hellerstein 1969) and triglycerides (Björntorp et al 1970 1972). It is *possible that the training in the present series* was not intensive enough to bring about the metabolic changes which training has been found to effect particularly in healthy subjects. The training must probably be nearly maximal before *its effects on serum lipids become manifest* Grimby et al (1975) observed that elderly men actively training and competing in orientation had significantly lower serum cholesterol and triglyceride values than a population of men of the same age. *Bjurö and Lundqvist (1975)* on the other hand, found no decline in cholesterol when training middle-aged men and only a slight decline in triglycerides The subjects trained were healthy 50-year-old men who had not previously engaged in physical activity It appears that not even a healthy person to say nothing of one recovering from myocardial infarction who begins physical activity during middle age is able to move so intensively that serum lipids would decline significantly which is probably why no decline of lipids could be recorded in the present work

Physical working capacity and subjective maximum. In the *first ergometric* test, the control men had a significantly higher mean PWC 130 than training men The groups did not *differ significantly* as regards the degree of the heaviness of work and free time physical activity prior to infarction. Since significantly more control men than training men lived in the country it is possible that the control men lived a physically more active life after discharge from *hospital This* is also suggested by the difference in the weekly amount of walking done by the training men and the *control men which was noted in the initial* checkup The difference may also have been a real difference in physical working capacity due to the living conditions, hypothesis an which is supported by the fact that the mean PWC 130 of the control

men was still higher at the end of the follow-up year than that of the training men.

No association of training with the increase of physical working capacity could be shown. Both the training subjects and the controls increased their mean PWC 130 significantly but the controls did so more. At the end of the year the mean PWC 130 of the training subjects was higher by 18.3 % than initially. The physical working capacity of 39.1 % of the trainers and 44.6 % of the controls improved to the normal level, i.e. by more than 20 %; the difference was not significant. The proportion of those who improved their physical working capacity by more than 10 % was also greater in the control group, though the difference was not significant. The physical working capacity of the training subjects improved more rapidly than that of the controls: in the training group neither the mean PWC 130, nor the proportion of those who improved their capacity by more than 10 % increased after the 3rd month, while in the control group both the mean PWC 130 and the proportion of subjects with over a 10 % improvement increased until the 6th month.

The mean PWC 130 of those who pedalled until their subjective maximum in the test increased more than that of the others. This may have been due to the fact that these patients got more encouragement for physical activity in the tests, after finding themselves to be capable of maximal exercise without any danger. Their spontaneous improvement was hence more effective than that of those who were strained submaximally in the tests.

According to Bruce et al (1973) the increase of aerobic capacity in coronary patients is mostly of a central origin. Since the mean PWC 130 of the training group did not increase relative to the controls, the training method utilized here probably did not have a significant central training effect.

It seems that the home training method employed in the present work does not yield equally as good results, in an unselected patient material, as attained in controlled studies with institutional rehabilitation (e.g. Saxne 1973). Absence of supervision may be a cause for ineffective training in many cases: the patients train too cautiously at home. During conducted and supervised training in an institute the variation in individual training effectiveness is smaller and the overall outcome of rehabilitation better. It is also possible that the chalisthenic effectiveness at home could have been improved by more strenuous training in the teaching sessions. It should be borne in mind, however, that several rehabilitation studies, where the aerobic capacity of infarction patients has been improved considerably, have been made on selected patient materials or with inadequate controls (e.g. Hellerstein 1968, Rechnitzer et al 1972) and the results do not correspond to the practical conditions in a clinic. Kentala (1972), in his well controlled study did not note any difference in the physical working capacity between the original training group and the control group.

The present work seems to suggest that home training can at least accelerate the recovery of aerobic capacity.

The subjective maximum of the training men increased significantly. At the end of the year it was 39.3 % higher than initially. The increase in the control group was 31.7 %, which was not significant. In the 9-month checkup, the subjective maximum of the training subjects was almost significantly higher than that of the controls. This indicates that some improvement of the physical condition took place in the training men. Since their mean PWC 130 values did not differ from those of the controls, and since the clinical findings did not change in comparison with the control men, the training effect was probably largely of peripheral origin. On the other hand, the training men apparently learnt to make more use of themselves during the follow-up period, and learnt to utilize their aerobic capacity better than the controls. This is suggested by the fact that the proportion of training men, interrupting the ergometric test because of chest pain, increased throughout the follow-up period, and the mean final pulse rate in the ergometric test was significantly higher in the group of training men than among the controls, from the 3rd month onwards.

The mean resting pulse rate, recorded prior to the ergometric test, was higher in the male training group from the 3rd month, and in the female training group from the 9th month onwards than in the control groups. The training subjects had always done some chalisthenic exercises prior to the ergometric test. Although a recovery time was allowed before the test, the normal pulse rate had apparently not been reached. This explains why the initial pulse rate of the trainers before the ergometric test became higher each time, as the chalisthenic exercises became more vigorous. The initial pulse rates of the trainers and the controls were hence not comparable.

Return to work. The training subjects and the controls were not significantly different in their return to work. The previous employment was resumed permanently by 37 training men (39 %), 35 control men (32 %), training women (26 %) and 5 control women (33 %). In addition, 3 training men and one control man moved into less strenuous occupation.

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R e t u r n t o w o r k. The training subjects and the controls were not significantly different in their return to work. The previous employment was resumed permanently by 37 training men (39 %), 35 control men (32 %), 7 training women (26 %) and 5 control women (23 %). In addition, 3 training men and one control man moved into a less strenuous occupation.

If compared with the results obtained in other countries, the figures for return to work are remarkably low Spontaneous return to work without rehabilitation in USA, the Soviet Union and Israel has been reported to be of the order of 80—90 % (Shapiro 1972, Gurevitch 1971) Kellerman and Kariv (1968) found 85 % of their rehabilitated patients returning to work. High figures for resumption of work have also been reported from Scandinavia (Malmcrona 1962, Björk 1964) Before the Law on Sickness Insurance came into force in Finland, over 60 % of those who had been capable of work before their illness returned to work after the first infarction (Iisalo 1958 Sipilä 1966) Once the Law on Sickness Insurance was enforced, return to work clearly decreased. According to Vuopala (1972) the percentage returning to work in Northern Finland was only 35 % Vuopala points out that the patient s occupation is crucial for return to work. Only 8 % of the smallholders resumed work, while 74 % of those with an academic degree did so Townspeople returned to work more frequently than country people

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In the present work the training subjects were followed up for an average of 31.5 months, and the controls for 26.5 months The control patients had more recidivous infarctions than the trainers A re infarction was suffered by 14.5 % of the controls and 11 7 % of the trainers. The difference was not significant. Coronary deaths were also more frequent among the controls than the training

subjects: 14 % of the controls and 10 % of the trainers died a coronary death. The difference as not significant. The time which elapsed before the coronary death was almost significantly longer among the trainers than in the control group (19.4 months and 11.8 months). Although the differences in the number of coronary deaths and recidivous infarctions ere not significant, it should be noted that both the training men and the training women had lower coronary mortality and recidivous morbidity than the controls. Training may have had some effect on morbidity and coronary mortality.

During the first year 3.5 % of the trainers and 8 % of the controls died. During the second year the corresponding figures were 3.6 % for the trainers and 5.5 % for the controls. During both the first and the second year the relative mortality in the training group was lower than that expected on the basis of the natural course of the disease. According to Zukel (1969), annual mortality from the second year onwards is 5 %.

Sudden deaths were equally numerous in the training group and the control group and equally many trainers and controls died in a state of physical activity. On the basis of this, training appears to have no effect on the number of sudden deaths.

Conclusions. Compared with supervised training in an institute, the type of conducted home training of infarction patients carried out in the present work appears to result in relatively little benefit. Home training improved the condition of infarction patients more rapidly than spontaneous recovery. There were also indications that home training may improve the prognosis. Home training, like rehabilitative measures in general, appeared to improve the patient psychic condition, so that they dared to utilize their remaining physical capacity better. Although spontaneous controlled home training seems to be safe, and probably does not cause any inconvenience to the patients, it is questionable whether the routine use of such rehabilitation would be useful in an unselected patient material.

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a 13.3 % higher PWC 130 and the controls an 18.3 % higher PWC 130 than observed initially PWC 130 improved by over 20 % in 39.1 % of the trainers and 44.6 % of the controls. The physical working capacity improved more rapidly in the group of trainers than in the control group. Those training men however for whom the subjective maximum was determined, seemed to improve their physical working capacity more than the controls, for the subjective maximum of the training men improved significantly (39.3 %). The improvement among the control men was not significant (31.7 %). The training effect achieved by the training men was probably peripheral, and did not become manifest in the PWC 130 measurements. Rehabilitation had no effect on the return to work. Forty (42.1 %) of the training men recovered their working capacity and 37 (39 %) of these returned to their previous employment; the corresponding figures for the control men were 36 (33 %) and 35 (32.1 %). Seven (25.9 %) of the training women and 5 (33.3 %) of the control women resumed their previous employment.

The prognosis of the training subjects was followed for an average of 31.5 months and that of the controls for an average of 26.5 months. During the follow-up period 21 trainers (11 %) and 29 controls (14.5 %) suffered a re-infarction. There were 18 (10 %) coronary deaths in the training group and 28 (14 %) in the control group. During the first year 3.5 % of the trainers and 8 % of the controls died. During the second year mortality was 3.6 % in the training group and 5.5 % in the control group. The trainers and controls did not differ significantly as regards recidivous infarctions and coronary deaths. It should be noted, however that the differences in both recidivous morbidity and coronary mortality were always in the same direction: the trainers had lower values of recidivous morbidity and coronary mortality than the controls. Training may have had an effect on the prognosis.

Summary

The purpose of this work on patients with myocardial infarction was to find out whether physical rehabilitation based on spontaneous home training can be carried out in practice, and whether such rehabilitation brings about favourable changes in the symptoms, clinical findings and metabolic risk factors A further objective was to find out whether the physical condition of the patients is improved, and whether the rehabilitation has any effect on the prognosis and return to work

The training group consisted of 180 patients, 143 men and 37 women. The control group comprised 200 patients, 166 men and 34 women All the patients were of working age, (under 65 yrs) and all were treated in the Clinic of Internal Medicine University Central Hospital of Oulu. Mobilization was early the intramural infarctions were discharged on the 12th day and the transmural ones on the 16th day

Rehabilitation was started 10 weeks after the onset of infarction The training subjects came for a chalistenic session in groups of 5—7 once a month. During the session they learnt a 30-minute series of chalistenic movements under the instruction of a physiotherapist, and the supervision of a physician The chalistenic program was an interval type program, involving all the large muscle groups and including running on the spot. The training became progressively more strenuous every month The trainers were to perform the program every day at home. During each session in a gymnastic hall, the subjects took a bicycle ergometer test. The controls came for an ergometric test monthly The patients were examined in an outpatient department every third month The improvement of their physical condition was followed by determining the submaximal value of aerobic power measured as the work load at a heart rate of 130 per minute (Physical Working Capacity 130) Furthermore the subjective maximum of about one fourth of the trainers and controls was determined in the ergometric test.

The program turned out to be suitable After 6 months, 78 % of the training men and 65 % of the training women were still participating and 67 % of the training men and 62 % of the training women attended the last session after 12 months.

The chalistenic home program was carried out enthusiastically Of the training men who were still participating after 6 months, 55 % did their exercises on 6—7 days of the week, the corresponding figure for the training women being 70 % after a year 51 % of the men and 73 % of the women were still equally active. At the time of the 12 month checkup 15 % of the men and 14 % of the women did an insignificant amount of chalistenic exercises (0—2 times a week)

Rehabilitation had no effect on the clinical condition of the trainers for no significant differences were noted in the symptoms of coronary disease and the clinical findings between the training subjects and the controls. One indication of a possible favourable training effect was the decrease of prolonged chest pains in the male training group during the follow up period. The blood pressure of the training men and the training women declined, but the difference in comparison with the controls was not significant.

At the end of the follow up year the training men were less fatigued subjectively than the controls which may be indicative of a favourable psychic effect of rehabilitation Rehabilitation had no effect on the smoking habits.

There were no significant differences in the ECG findings between the trainers and the controls.

The relative heart volume of both the training men and the training women increased during the year In the initial checkup the mean relative heart volume of the training men was 496 cm^3/m^2 and that of the training women 487 cm^3/m^2 while at the end of the year the figures were 506 cm^3/m^2 for men and 530 cm^3/m^2 for women. The difference between the trainers and the controls was almost significant in both groups at the end of the year The clinical findings in the training group showed no signs of an increase of heart failure at the end of the year Rehabilitation had no effect on serum cholesterol serum triglycerides or serum urate or 2 hour glucose tolerance

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Appendix

Table I. Patients

	Training subjects	Controls	Total
Men	143	166	309
Women	37	34	71
Total	180	200	380

Table II. Age; mean values and standard deviations

	Training men	Control men	Training women	Control women
Mean	52.4	51.6	54.7	57.4
S.D.	7.7	8.5	7.2	6.7
	143	166	37	34

Table III. Marital status

	Training men	Control men	Training women	Control women
Unmarried	7 (4.9 %)	13 (7.8 %)	4 (10.8 %)	1 (.9 %)
Married	127 (88.8 %)	145 (87.4 %)	25 (67.6 %)	21 (61.8 %)
Widowed	4 (2.8 %)	3 (1.8 %)	6 (16.2 %)	11 (32.4 %)
Divorced	5 (3.5 %)	5 (3.0 %)	2 (5.4 %)	1 (2.9 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table IV. Place of residence

	Training men	Control men	Training women	Control women
Country	62 (43.4 %)	100* (60.2 %)	21 (56.8 %)	17 (50 %)
Town	81 (56.6 %)	66 (39.8 %)	16 (43.2 %)	17 (50 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

$p < 0.001$

Table V. Occupational distribution

	Training men	Control men	Training women	Control women
Unskilled labourers	20 (14.0 %)	22 (13.3 %)	10 (27.0 %)	1 (2.9 %)
Smallholders, lumberjacks	10 (7.0 %)	18 (10.8 %)	3 (8.1 %)	—
Farmers	22 (15.4 %)	33 (19.9 %)	9 (24.3 %)	12 (35.3 %)
Skilled employees in commerce	21 (14.7 %)	18 (10.8 %)	5 (13.5 %)	2 (5.9 %)
Skilled industrial workers	36 (25.2 %)	31 (18.7 %)	—	2 (5.9 %)
Supervisors, foremen	2 (1.4 %)	6 (3.6 %)	—	—
Small-scale businessmen	15 (10.5 %)	20 (12.1 %)	—	2 (5.9 %)
Civil servants	11 (7.7 %)	11 (6.6 %)	2 (5.4 %)	2 (5.9 %)
Managerial position academic degree	6 (4.2 %)	6 (3.6 %)	—	—
Housewives, house owners	—	1 (0.6 %)	8 (21.6 %)	13 (38.2 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

$p < 0.05$

Table VI Physical activity in work before present myocardial infarction (MI)

	Training men	Control men	Training women	Control women
Sedentary work	28 (19 6 %)	36 (21 7 %)	2 (5 4 %)	3 (8.8 %)
Walking during work	45 (31.5 %)	38 (22.9 %)	17 (46.0 %)	22 (64 7 %)
Much walking liftings	32 (22.4 %)	33 (19 9 %)	15 (40.5 %)	9 (26.5 %)
Heavy manual work	38 (26 6 %)	59 (35.5 %)	3 (8.1 %)	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table VII Working capacity before MI

	Training men	Control men	Training women	Control women
Able to work	95 (66 4 %)	109 (65 7 %)	27 (73 0 %)	15 (44 1 %)
Sickness pension	39 (27.3 %)	45 (27 1 %)	9 (24.3 %)	14 (41.2 %)
Employment pension	1 (0 7 %)	1 (0.6 %)	—	2 (5 9 %)
Sick leave	8 (5 6 %)	11 (6.6 %)	1 (2.7 %)	3 (8.8 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table VIII Reason for disability to work before MI

	Training men	Control men	Training women	Control women
Previous MI	17 (36.2 %)	20 (35 7 %)	4 (40.0 %)	7 (41.2 %)
CHD without MI	18 (38.3 %)	23 (41 1 %)	5 (50.0 %)	8 (47 1 %)
Other reason	12 (25.5 %)	13 (23.2 %)	1 (10 0 %)	2 (11.8 %)
Total	47 (100 %)	56 (100 %)	10 (100 %)	17 (100 %)

Table IX. Physical activity in leisure time before MI

	Training men	Control men	Training women	Control women
Fully sedentary	97 (67.8 %)	113 (68 1 %)	30 (81 1 %)	27 (79 4 %)
Walking, cycling at least 4 hours/week	44 (30.8 %)	47 (28.3 %)	7 (18.9 %)	7 (20 6 %)
Running, swimming etc 3 hours/week	1 (0 7 %)	6 (3 6 %)	—	—
Active training regularly several times/week	1 (0 7 %)	—	—	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table X. Number of present myocardial infarction (MI)

	Training men	Control men	Training women	Control women
First MI	113 (79 0 %)	132 (79.5 %)	33 (89.2 %)	27 (79 4 %)
Second MI	22 (15 4 %)	24 (14.5 %)	4 (10.8 %)	5 (14 7 %)
Third MI	7 (4 9 %)	7 (4.2 %)	—	2 (5 9 %)
Fourth MI	1 (0 7 %)	1 (0 6 %)	—	—
Fifth MI	—	2 (1 2 %)	—	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XI. Previous symptoms

	Training men	Control men	Training women	Control women
Angina pectoris	52 (36.4 %)	66 (39.8 %)	16 (43.2 %)	7 (20.6 %)
Dyspnea	6 (4.2 %)	4 ([illegible].4 %)	3 (8.1 %)	3 (8.8 %)
Palpitation	2 (1.4 %)	—	—	2 (5.9 %)
Angina pectoris + dyspnea	50 (35.0 %)	64 (38.6 %)	14 (37.8 %)	19 (55.9 %)
Angina pectoris + palpitation	4 (2.8 %)	1 (0.6 %)	1 (2.7 %)	—
Dyspnea + palpitation	—	—	—	—
Angina pectoris + dyspn + palpit.	—	1 (0.6 %)	—	—
Asymptomatic	28 (19.4 %)	29 (17.5 %)	3 (8.1 %)	3 (8.8 %)
MI — later asymptomatic	1 (0.7 %)	1 (0.6 %)	—	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XII. Duration of previous symptoms

	Training men	Control men	Training women	Control women
< 3 months	12 (10.4 %)	21 (15.3 %)	4 (11.8 %)	—
3—6 months	9 (7.8 %)	10 (7.3 %)	2 (5.9 %)	1 (3.2 %)
6—12 months	4 (3.5 %)	15 (11.0 %)	4 (11.8 %)	—
1—3 years	34 (29.6 %)	36 (26.3 %)	6 (17.7 %)	(16.1 %)
3—5 years	25 (21.7 %)	18 (13.1 %)	6 (17.7 %)	10 (32.3 %)
5—10 years	21 (18.3 %)	22 (16.1 %)	7 (20.6 %)	9 (29.0 %)
> 10 years	10 (8.7 %)	15 (11.0 %)	5 (14.7 %)	6 (19.4 %)
Total	115 (100 %)	137 (100 %)	34 (100 %)	31 (100 %)

Table XIII. Time which elapsed before admission into hospital

	Training men	Control men	Training women	Control women
Less than one day	116 (81.1 %)	129 (77.7 %)	32 (86.5 %)	28 (82.4 %)
One day	17 (11.9 %)	16 (9.6 %)	3 (8.1 %)	4 (11.8 %)
Two days	2 (1.4 %)	7 (4.2 %)	2 (5.4 %)	1 (2.9 %)
Three days	2 (1.4 %)	4 (2.4 %)	—	—
More than three days	6 (4.2 %)	10 (6.0 %)	—	1 (2.9 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XIV. Extent of infarction, based on ECG

	Training men	Control men	Training women	Control women
Intramural	54 (37.8 %)	68 (41.0 %)	19 (51.4 %)	16 (47.1 %)
Transmural	89 (62.2 %)	98 (59.0 %)	18 (48.7 %)	18 (52.9 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XV. Complicated infarctions

	Training men	Control men	Training women	Control women
Complicated	15 (10.5 %)	23 (13.9 %)	7 (18.9 %)	5 (14.7 %)
Not complicated	128 (89.5 "~)	143 (86.1 %)	30 (81.1 %)	29 (85.3 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table VI Physical activity in work before present myocardial infarction (MI)

	Training men	Control men	Training women	Control women
Sedentary work	28 (19 6 %)	36 (21 7 %)	2 (5.4 %)	3 (8.8 %)
Walking during work	45 (31.5 %)	38 (22.9 %)	17 (46.0 %)	22 (64.7)
Much walking, liftings	32 (22.4 %)	33 (19 9 %)	15 (40.5 %)	9 (26.5 %)
Heavy manual work	38 (26.6 %)	59 (35.5 %)	3 (8.1 %)	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table VII Working capacity before MI

	Training men	Control men	Training women	Control women
Able to work	95 (66 4 %)	109 (65 7 %)	27 (73 0 %)	15 (44.1)
Sickness pension	39 (27.3 %)	45 (27 1 %)	9 (24.3 %)	14 (41.2 %)
Employment pension	1 (0.7 %)	1 (0 6 %)	—	2 (5.9 %)
Sick leave	8 (5.6 %)	11 (6.6 %)	1 (2.7 %)	3 (8.8 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table VIII Reason for disability to work before MI

	Training men	Control men	Training women	Control women
Previous MI	17 (36.2 %)	20 (35 7 %)	4 (40.0 %)	7 (41.2 %)
CHD without MI	18 (38.3 %)	23 (41 1 %)	5 (50.0 %)	8 (47 1 %)
Other reason	12 (25.5 %)	13 (23.2 %)	1 (10 0 %)	2 (11.8 %)
Total	47 (100 %)	56 (100 %)	10 (100 %)	17 (100 %)

Table IX Physical activity in leisure time before MI

	Training men	Control men	Training women	Control women
Fully sedentary	97 (67.8 %)	113 (68.1 %)	30 (81 1 %)	27 (79 4 %)
Walking, cycling at least 4 hours/week	44 (30.8 %)	47 (28.3 %)	7 (18.9 %)	7 (20.6 %)
Running swimming etc 3 hours/week	1 (0 7 %)	6 (3 6 %)	—	—
Active training regularly several times/week	1 (0 7 %)	—	—	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table X. Number of present myocardial infarction (MI)

	Training men	Control men	Training women	Control women
First MI	113 (79.0 %)	132 (79.5 %)	33 (89.2 %)	27 (79 4 %)
Second MI	22 (15 4 %)	24 (14.5 %)	4 (10.8 %)	5 (14 7 %)
Third MI	7 (4 9 %)	7 (4.2 %)	—	2 (5 9 %)
Fourth MI	1 (0.7 %)	1 (0.6 %)	—	—
Fifth MI	—	2 (1.2 %)	—	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XX. Mean cholesterol time (minutes) at each training spell at home

	Training men	Training women
1—2 months	37.1 ± 18.3	38.0 ± 16.2
2—3 — —	36.9 ± 18.3	41.3 ± 21.6
3—4 — —	33.9 ± 18.1	37.8 ± 21.1
4—5 — —	35.4 ± 17.3	35.6 ± 18.1
5—6 — —	35.4 ± 18.8	40.7 ± 19.8
6—9 — —	32.3 ± 18.6	37.0 ± 22.9
9—12 — —	34.2 ± 20.7	34.3 ± 14.9

Table XXI. Skill of the participants in supervised cholesterol analysis graded with 1—3 scale

A. Training men

	2 months	3 months	4 months	5 months	6 months	9 months	12 months
1	8 (6.8 %)	6 (5.6 %)	9 (7.8 %)	9 (8.0 %)	7 (6.3 %)	4 (3.8 %)	3 ([illegible] %)
2	67 (56.8 %)	66 (61.1 %)	61 (52.6 %)	64 (57.1 %)	68 (60.7 %)	66 (63.5 %)	59 (62.1 %)
3	43 (36.4 %)	36 (33.3 %)	46 (39.7 %)	39 (34.8 %)	37 (33.0 %)	34 (32.7 %)	35 ([illegible] %)
Total	118 (100 %)	108 (100 %)	116 (100 %)	112 (100 %)	112 (100 %)	104 (100 %)	95 (100 %)

B. Training women

	2 months	3 months	4 months	5 months	6 months	9 months	12 months
1	5 (18.5 %)	3 (13.0 %)	7 (28.0 %)	2 (8.0 %)	4 (16.7 %)	2 (8.3 %)	2 (8.7 %)
2	16 (59.3 %)	12 (52.2 %)	13 (52.0 %)	16 (64.0 %)	16 (66.7 %)	14 (58.3 %)	16 (69.6 %)
3	6 (22.2 %)	8 (34.8 %)	5 (20.0 %)	7 (28.0 %)	4 (16.7 %)	8 (33.3 %)	5 (21.7 %)
Total	27 (100 %)	23 (100 %)	25 (100 %)	25 (100 %)	24 (100 %)	24 (100 %)	23 (100 %)

Table XXII. Frequency of angina pectoris attacks

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No angina pectoris	27 (18.9 %)	24 (15.7 %)	26 (19.5 %)	25 (16.6 %)	22 (17.3 %)	21 (14.5 %)	17 (13.8 %)	20 (14.2 %)	16 (13.2 %)	19 (13.7 %)
Infrequently	17 (11.9 %)	14 (8.4 %)	14 (10.5 %)	9 (6.0 %)	15 (11.8 %)	11 (7.6 %)	11 (8.9 %)	11 (7.8 %)	12 (9.9 %)	11 (7.9 %)
Monthly	1 (0.7 %)	6 (3.6 %)	9 (6.8 %)	15 (9.9 %)	10 (7.9 %)	15 (10.3 %)	15 (12.2 %)	11 (7.8 %)	22 (18.2 %)	14 (10.1 %)
Weekly	58 (40.6 %)	67 (40.4 %)	49 (36.8 %)	57 (37.7 %)	45 (35.4 %)	65 (44.8 %)	49 (39.8 %)	55 (39.0 %)	44 (36.4 %)	57 (41.0 %)
Daily	40 (28.0 %)	53 (31.9 %)	35 (26.3 %)	45 (29.8 %)	35 (27.6 %)	33 (22.8 %)	31 (25.2 %)	44 (31.2 %)	27 (22.3 %)	38 (27.3 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No angina pectoris	7 (18.9 %)	4 (11.8 %)	6 (17.1 %)	5 (14.7 %)	5 (15.6 %)	5 (15.6 %)	4 (12.9 %)	3 (10.3 %)	4 (14.3 %)	3 (10.3 %)
Infrequently	5 (13.5 %)	3 (8.8 %)	3 (8.6 %)	3 (8.8 %)	3 (9.4 %)	3 (9.4 %)	3 (9.7 %)	6 (20.7 %)	2 (7.1 %)	3 (10.3 %)
Monthly	1 (2.7 %)	3 (8.8 %)	3 (8.6 %)	4 (11.8 %)	2 (6.3 %)	4 (12.5 %)	3 (9.7 %)	4 (13.8 %)	6 (21.4 %)	6 (20.7 %)
Weekly	15 (40.5 %)	14 (41.2 %)	12 (34.3 %)	11 (32.4 %)	13 (40.6 %)	12 (37.5 %)	16 (51.6 %)	10 (34.5 %)	11 (39.3 %)	9 (31.0 %)
Daily	9 (24.3 %)	10 (29.4 %)	11 (31.4 %)	11 (32.4 %)	9 (28.1 %)	8 (25.0 %)	5 (16.1 %)	6 (20.7 %)	5 (17.9 %)	8 (27.6 %)
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XVI Localization of infarction, based on ECG

	Training men	Control men	Training women	Control women
Anterior	77 (53 9 %)	88 (53.0 %)	26 (70.3 %)	25 (73.5 %)
Posterior	62 (43 4 %)	72 (43 4 %)	9 (24.3 %)	9 (26.5 ")
Not known	4 (2.8 %)	6 (3 6 %)	2 (5 4 ")	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XVII Anterior infarctions

	Training men	Control men	Training women	Control women
Anteroseptal	43 (55.8 %)	51 (58 6 %)	9 (34 6 %)	12 (48.0 ")
Other	34 (44 2 %)	37 (41 4 %)	17 (65.4 %)	13 (52.0 %)
Total	77 (100 %)	88 (100 %)	26 (100 %)	25 (100 %)

Table XVIII Participation in supervised chalistenic sessions and checkups in the outpatient department

	Training men		Training women		Control men	Control women
	Chalistenic session	Outpatient department	Chalistenic session	Outpatient department	Outpatient department	
Initial checkup		143 (100 %)		37 (100 %)	166 (100 %)	34 (100 ")
1st month	131 (91 0 %)		33 (89 2 %)		163 (98.2 %)	33 (97 1 %)
2nd — —	121 (84 7 %)		27 (73 0 %)		155 (93 4 %)	32 (94.2 ")
3rd — —	111 (77 7 %)	134 (93.8 %)	23 (62.1 %)	35 (94 6 %)	151 (91 0 %)	34 (100 %)
4th — —	118 (82.5 %)		25 (67 6 %)		141 (85.0 %)	32 (94 2 ")
5th — —	112 (78 3 %)		25 (67 6 %)		138 (83.1 %)	32 (94.2 ")
6th — —	112 (78.3 %)	127 (88 8 %)	24 (64 9 %)	32 (86.5 %)	145 (87 4 %)	32 (94 2 ")
9th — —	104 (72.8 %)	123 (86.0 %)	24 (64 9 %)	31 (83 8 %)	141 (85 0 %)	29 (85.4 ")
12th — —	96 (67.2 %)	121 (84 7 %)	23 (62.1 %)	28 (75 7 %)	139 (83.8 ")	29 (85 4 ")

Table XIX. Chalistenic activity at home; amount of chalistenic exercises performed/week

A Training men

	1—2 months	2—3 months	3—4 months	4—5 months	5—6 months	6—9 months	9—12 months
1	2 (1 7 %)	—	2 (1 7 %)	6 (5 4 %)	6 (5 4 %)	2 (1 9 %)	2 (2.1 %)
2	4 (3.3 %)	8 (7.2 %)	6 (5 1 %)	5 (4.5 %)	5 (4.5 %)	6 (5.8 ")	9 (9 4 ")
3	8 (6 6 %)	10 (9 0 %)	7 (5 9 %)	5 (4.5 %)	9 (8.0 %)	6 (5.8 %)	8 (8.3 ")
4	13 (10 7 %)	14 (12.6 %)	11 (9.3 %)	13 (11 6 %)	16 (14.3 %)	14 (13.5 %)	15 (15.6 ")
5	12 (9 9 %)	8 (7 2 %)	14 (11 9 %)	15 (13 4 %)	14 (12.5 %)	12 (11.5 ")	12 (12.5 ")
6	35 (28 9 %)	25 (22.5 %)	25 (21.2 %)	21 (18.8 %)	25 (22.3 %)	28 (26 9 ")	17 (17 7 %)
7	45 (37 1 %)	44 (39 6 %)	48 (40 7 %)	46 (41 1 %)	35 (31.3 %)	34 (32.7 %)	30 (31.3 %)
0	2 (1 7 %)	2 (1.8 %)	5 (4 2 %)	1 (0 9 %)	2 (1.8 %)	2 (1 9 %)	3 (3 1 ")
Total	121 (100 %)	111 (100 %)	118 (100 %)	112 (100 %)	112 (100 %)	104 (100 %)	96 (100 ")

B Training women

	1—2 months	2—3 months	3—4 months	4—5 months	5—6 months	6—9 months	9—12 months
1	—	—	2 (8.0 %)	4 (16.0 ")	1 (4 2 ")	2 (8 3 ")	1 (4 3 ")
2	1 (3 7 %)	1 (4 3 %)	2 (8.0 %)	—	2 (8.3 %)	3 (12 5 ")	1 (4 3 ")
3	2 (7 4 %)	—	2 (8 0 %)	1 (4.0 %)	1 (4 2 ")	—	—
4	6 (22.2 %)	3 (13.0 %)	2 (8.0 %)	—	2 (8.3 ")	2 (8.3 ")	1 (4 3 ")
5	4 (14 8 %)	1 (4.3 %)	3 (12.0 %)	4 (16.0 ")	1 (4 2 %)	3 (12.5 ")	3 (13 0)
6	6 (22.2 %)	7 (30 4 %)	4 (16.0 %)	8 (32.0 ")	5 (20 8 ")	5 (20 8 ")	6 (26 1 ")
7	8 (29 6 ")	8 (34 8 %)	9 (36.0 %)	8 (32.0 ")	11 (45.8 ")	9 (37.5 ")	10 (43.5 ")
0	—	3 (13 0 %)	1 (4 0 %)	—	1 (4 2 ")	—	1 (4 3 ")
Total	27 (100 ")	23 (100 %)	25 (100 ")	25 (100 ")	24 (100 ")	24 (100 ")	23 (100 ")

Table XX. Mean callisthenic time (minutes) in each training spell at home

	Training men	Training women
1—2 months	37.1 ± 18.3	38.0 ± 16.2
2—3 — —	36.9 ± 18.3	41.3 ± 23.6
3—4 — —	37.9 ± 18.1	37.8 ± 21.1
4—5 — —	35.4 ± 17.3	35.6 ± 18.1
5—6 — —	35.4 ± 18.8	40.7 ± 19.8
6—9 — —	32.3 ± 18.6	37.0 ± 22.9
9—12 — —	34.2 ± 20.7	34.3 ± 14.9

Table XXI. Skill of the participants in supervised callisthenic sessions graded with 1—3 scale

A. Training men

	2 months	3 months	4 months	5 months	6 months	9 months	12 months
1	8 (6.8 %)	6 (5.6 %)	9 (7.8 %)	9 (8.0 %)	7 (6.3 %)	4 (3.8 %)	1 (1.1 %)
2	67 (56.8 %)	66 (61.1 %)	61 (52.6 %)	64 (57.1 %)	68 (60.7 %)	66 (63.5 %)	59 (62.1 %)
3	43 (36.4 %)	36 (33.3 %)	46 (39.7 %)	39 (34.8 %)	37 (33.0 %)	34 (32.7 %)	35 (36.8 %)
Total	118 (100 %)	108 (100 %)	116 (100 %)	112 (100 %)	112 (100 %)	104 (100 %)	95 (100 %)

B. Training women

	2 months	3 months	4 months	5 months	6 months	9 months	12 months
1	5 (18.5 %)	3 (13.0 %)	7 (28.0 %)	2 (8.0 %)	4 (16.7 %)	2 (8.3 %)	2 (8.7 %)
2	16 (59.3 %)	12 (52.2 %)	13 (52.0 %)	16 (64.0 %)	16 (66.7 %)	14 (58.3 %)	16 (69.6 %)
3	6 (22.2 %)	8 (34.8 %)	5 (20.0 %)	7 (28.0 %)	4 (16.7 %)	8 (33.3 %)	5 (21.7 %)
Total	27 (100 %)	23 (100 %)	25 (100 %)	25 (100 %)	24 (100 %)	24 (100 %)	23 (100 %)

Table XXII. Frequency of angina pectoris attacks

A. Men

	Initial checkup		3 month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No angina pectoris	27 (18.9 %)	26 (15.7 %)	26 (19.5 %)	25 (16.6 %)	22 (17.3 %)	21 (14.5 %)	17 (13.8 %)	20 (14.2 %)	14 (11.2 %)	19 (13.7 %)
Infrequently	17 (11.9 %)	14 (8.4 %)	14 (10.5 %)	9 (6.0 %)	15 (11.8 %)	11 (7.6 %)	11 (8.9 %)	11 (7.8 %)	12 (9.9 %)	11 (7.9 %)
Monthly	1 (0.7 %)	6 (3.6 %)	9 (6.8 %)	15 (9.9 %)	10 (7.9 %)	15 (10.3 %)	15 (12.2 %)	11 (7.8 %)	22 (18.2 %)	14 (10.1 %)
Weekly	58 (40.6 %)	67 (40.4 %)	49 (36.8 %)	57 (37.7 %)	45 (35.4 %)	65 (44.8 %)	49 (39.8 %)	55 (39.0 %)	44 (36.4 %)	57 (41.0 %)
Daily	40 (28.0 %)	53 (31.9 %)	35 (26.3 %)	45 (29.8 %)	35 (27.6 %)	33 (22.8 %)	31 (25.2 %)	44 (31.2 %)	27 (22.3 %)	38 (27.3 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No angina pectoris	7 (18.9 %)	4 (11.8 %)	6 (17.1 %)	5 (14.7 %)	5 (15.6 %)	5 (15.6 %)	4 (12.9 %)	3 (10.3 %)	4 (14.3 %)	3 (10.3 %)
Infrequently	5 (13.5 %)	3 (8.8 %)	3 (8.6 %)	3 (8.8 %)	3 (9.4 %)	3 (9.4 %)	3 (9.7 %)	6 (20.7 %)	2 (7.1 %)	3 (10.3 %)
Monthly	1 (2.7 %)	3 (8.8 %)	3 (8.6 %)	4 (11.8 %)	2 (6.3 %)	4 (12.5 %)	3 (9.7 %)	4 (13.8 %)	6 (21.4 %)	6 (20.7 %)
Weekly	15 (40.5 %)	14 (41.2 %)	12 (34.3 %)	11 (32.4 %)	13 (40.6 %)	12 (37.5 %)	16 (51.6 %)	10 (34.5 %)	11 (39.3 %)	9 (31.0 %)
Daily	9 (24.3 %)	10 (29.4 %)	11 (31.4 %)	11 (32.4 %)	9 (28.1 %)	8 (25.0 %)	5 (16.1 %)	6 (20.7 %)	5 (17.9 %)	8 (27.6 %)
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XVI Localization of Infarction based on ECG

	Training men	Control men	Training women	Control women
Anterior	77 (53 9 %)	88 (53 0 %)	26 (70.3 %)	25 (73.5 %)
Posterior	62 (43 4 %)	72 (43 4 %)	9 (24.3 %)	9 (26.5 %)
Not known	4 (2.8 %)	6 (3 6 %)	2 (5 4 %)	—
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table XVII Anterior Infarctions

	Training men	Control men	Training women	Control women
Anteroseptal	43 (55.8 %)	51 (58 6 %)	9 (34 6 %)	12 (48.0 %)
Other	34 (44.2 %)	37 (41 4 %)	17 (65.4 %)	13 (52.0 %)
Total	77 (100 %)	88 (100 %)	26 (100 %)	25 (100 %)

Table XVIII Participation in supervised chalistenic sessions and checkups in the outpatient department

	Training men		Training women		Control men	Control women
	Chalistenic session	Outpatient department	Chalistenic session	Outpatient department	Outpatient department	
Initial checkup		143 (100 %)		37 (100 %)	166 (100 %)	34 (100 %)
1st month	131 (91 0 %)		33 (89 2 %)		163 (98.2 %)	33 (97 1 %)
2nd — —	121 (84 7 %)		27 (73 0 %)		155 (93 4 %)	32 (94.2 %)
3rd — —	111 (77 7 %)	134 (93 8 %)	23 (62.1 %)	35 (94 6 %)	151 (91 0 %)	34 (100 %)
4th — —	118 (82.5 %)		25 (67 6 %)		141 (85 0 %)	32 (94 2 %)
5th — —	112 (78 3 %)		25 (67 6 %)		138 (83 1 %)	32 (94.2 %)
6th — —	112 (78.3 %)	127 (88.8 %)	24 (64 9 %)	32 (86.5 %)	145 (87 4 %)	32 (94 2 %)
9th — —	104 (72.8 %)	123 (86 0 %)	24 (64 9 %)	31 (83.8 %)	141 (85 0 %)	29 (85 4 %)
12th — —	96 (67 2 %)	121 (84 7 %)	23 (62.1 %)	28 (75 7 %)	139 (83 8 %)	29 (85 4 %)

Table XIX Chalistenic activity at home; amount of chalistenic exercises performed/week

A. Training men

	1—2 months	2—3 months	3—4 months	4—5 months	5—6 months	6—9 months	9—12 months
1	2 (1 7 %)	—	2 (1 7 %)	6 (5 4 %)	6 (5 4 %)	2 (1 9 %)	2 (2.1 %)
2	4 (3.3 %)	8 (7 2 %)	6 (5 1 %)	5 (4.5 %)	5 (4.5 %)	6 (5 8 %)	9 (9 4 %)
3	8 (6 6 %)	10 (9 0 %)	7 (5 9 %)	5 (4.5 %)	9 (8.0 %)	6 (5 8 %)	8 (8.3 %)
4	13 (10 7 %)	14 (12.6 %)	11 (9.3 %)	13 (11 6 %)	16 (14.3 %)	14 (13.5 %)	15 (15 6 %)
5	12 (9 9 %)	8 (7 2 %)	14 (11 9 %)	15 (13 4 %)	14 (12.5 %)	12 (11.5 %)	12 (12.5 %)
6	35 (28.9 %)	25 (22.5 %)	25 (21.2 %)	1 (18 8 %)	25 (22.3 %)	28 (26 9 %)	17 (17 7 %)
7	45 (37 1 %)	44 (39 6 %)	48 (40 7 %)	46 (41 1 %)	35 (31.3 %)	34 (32.7 %)	30 (31.3 %)
0	2 (1 7 %)	2 (1.8 %)	5 (4 2 %)	1 (0.9 %)	2 (1 8 %)	2 (1 9 %)	3 (3 1 %)
Total	121 (100 %)	111 (100 %)	118 (100 %)	112 (100 %)	112 (100 %)	104 (100 %)	96 (100 %)

B. Training women

	1—2 months	2—3 months	3—4 months	4—5 months	5—6 months	6—9 months	9—12 months
1	—	—	2 (8.0 %)	4 (16 0 %)	1 (4.2 %)	2 (8.3 %)	1 (4 3 %)
2	1 (3 7 %)	1 (4.3 %)	2 (8.0 %)	—	2 (8 3 %)	3 (12.5 %)	1 (4 3 %)
3	2 (7 4 %)	—	2 (8.0 %)	1 (4 0 %)	1 (4 2 %)	—	—
4	6 (22.2 %)	3 (13.0 %)	2 (8.0 %)	—	2 (8.3 %)	2 (8.3 %)	1 (4 3)
5	4 (14 8 %)	1 (4.3 %)	3 (12 0 %)	4 (16.0 %)	1 (4 2 %)	3 (12.5 %)	3 (13 0 %)
6	6 (22.2 %)	7 (30.4 %)	4 (16 0 %)	8 (32.0 %)	5 (20.8 %)	5 (20 8 %)	6 (26 1)
7	8 (29 6 %)	8 (34.8 %)	9 (36 0 %)	8 (32.0 %)	11 (45 8 %)	9 (3 .5 %)	10 (43 5 %)
0	—	3 (13 0 %)	1 (4 0 %)	—	1 (4 2 %)	—	1 (4 3 %)
Total	27 (100 %)	23 (100 %)	.5 (100 %)	25 (100 %)	24 (100 %)	24 (100 %)	23 (100 %)

Table XXIV Duration of angina pectoris attacks

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Minutes	98 (84.5 %)	108 (77.1 %)	86 (80.4 %)	94 (74.6 %)	82 (78.1 %)	83 (66.9 %)	84 (79.2 %)	79 (65.3 %)	84 (80.0 %)	88 (73.3 %)
1/2 hour	4 (3.5 %)	10 (7.1 %)	1 (0.9 %)	4 (3.2 %)	6 (5.7 %)	7 (5.6 %)	5 (4.7 %)	6 (5.0 %)	1 (1.0 %)	5 (4.2 %)
Hours	8 (6.9 %)	8 (5.7 %)	6 (5.6 %)	8 (6.3 %)	6 (5 %)	11 (8.9 %)	6 (5.7 %)	9 (7.4 %)	4 (3.7 %)	10 (8.3 %)
Min. + 1/2 h	1 (0.9 %)	4 (2.9 %)	3 (2.8 %)	2 (1.6 %)	1 (1.0 %)	6 (4.8 %)	1 (0.9 %)	4 (3.3 %)	3 (2.9 %)	3 (2.5 %)
Min. + hs	5 (4.3 %)	7 (5.0 %)	9 (8.4 %)	15 (11.9 %)	8 (7.6 %)	11 (8.9 %)	7 (6.6 %)	18 (14.9 %)	8 (7.6 %)	10 (8.3 %)
1/2 h + hs	—	—	1 (0.9 %)	1 (0.8 %)	2 (1.9 %)	4 (3.2 %)	1 (0.9 %)	3 (2.5 %)	2 (1.9 %)	3 (2.5 %)
Min. + 1/2h + hs	—	3 (2.1 %)	1 (0.9 %)	2 (1.6 %)	—	2 (1.6 %)	2 (1.9 %)	2 (1.7 %)	1 (1.0 %)	1 (0.8 %)
Total	116 (100 %)	140 (100 %)	107 (100 %)	126 (100 %)	105 (100 %)	124 (100 %)	106 (100 %)	121 (100 %)	105 (100 %)	120 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Minutes	22 (73.3 %)	26 (86.7 %)	23 (79.3 %)	24 (82.8 %)	24 (88.9 %)	21 (77.8 %)	19 (70.4 %)	22 (84.6 %)	19 (79.2 %)	18 (69.2 %)
1/2 hour	4 (13.3 %)	1 (3.3 %)	3 (10.3 %)	1 (3.4 %)	—	1 (3.7 %)	3 (11.1 %)	—	1 (4.2 %)	—
Hours	1 (3.3 %)	1 (3.3 %)	1 (3.4 %)	1 (3.4 %)	1 (3.7 %)	1 (3.7 %)	2 (7.4 %)	1 (3.8 %)	2 (8.3 %)	2 (7.7 %)
Min. + 1/2 h	—	—	—	1 (3.4 %)	1 (3.7 %)	1 (3.7 %)	1 (3.7 %)	1 (3.8 %)	—	3 (11.5 %)
Min. + hs	1 (3.3 %)	2 (6.7 %)	1 (3.4 %)	1 (3.4 %)	1 (3.7 %)	3 (11.1 %)	1 (3.7 %)	2 (7 %)	2 (8.3 %)	3 (11.5 %)
1/2 h + hs	1 (3.3 %)	—	—	1 (3.4 %)	—	—	1 (3.7 %)	—	—	—
Min. + 1/2h + hs	1 (3.3 %)	—	1 (3.4 %)	—	—	—	—	—	—	—
Total	30 (100 %)	30 (100 %)	29 (100 %)	29 (100 %)	27 (100 %)	27 (100 %)	27 (100 %)	26 (100 %)	24 (100 %)	26 (100 %)

Table XXIII Causes of angina pectoris attacks

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 116)	Control group (n = 140)	Training group (n = 107)	Control group (n = 126)	Training group (n = 105)	Control group (n = 124)	Training group (n = 106)	Control group (n = 121)	Training group (n = 105)	Control group (n = 120)
Use of hands	72 (62.1 %)	94 (67 1 %)	86 (80 4 %)	102 (81 0 %)	86 (81 9 %)	108 (87 1 %)	80 (75.5 %)	108 (89.3 %)**	81 (77 1 %)	107 (89.2 %)*
Walking	86 (74 1 %)	112 (80 0 %)	83 (77 6 %)	102 (81 0 %)	84 (80.0 %)	102 (82.9 %)	79 (74.5 %)	101 (83.5 %)	82 (78.1 %)	97 (80.8 %)
Excitement	70 (60.3 %)	90 (64.3 %)	69 (64.5 %)	91 (72.2 %)	79 (75.2 %)	94 (75.8 %)	77 (72.6 %)	91 (75 2 %)	77 (73.3 %)	85 (70.8 %)
Chest pains in sleep	29 (25.0 %)	48 (34.3 %)	26 (24.3 %)	48 (38.1 %)*	30 (28 6 %)	50 (40 3 %)	29 (27 4 %)	57 (47 1 %)	40 (38.1 %)	59 (49.2 %)
Other cause	15 (12.9 %)	20 (14.3 %)	11 (10.3 %)	20 (15 9 %)	8 (7 6 %)	18 (14.5 %)	19 (17 9 %)	15 (12.4 %)	15 (14 3 %)	11 (9 2 %)

B Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 30)	Control group (n = 30)	Training group (n = 29)	Control group (n = 29)	Training group (n = 27)	Control group (n = 27)	Training group (n = 27)	Control group (n = 26)	Training group (n = 24)	Control group (n = 26)
Use of hands	19 (63 3 %)	23 (76.7 %)	21 (72.4 %)	28 (96 6 %)	22 (68.8 %)	27 (84 4 %)	24 (88 9 %)	22 (84 6 %)	21 (87.5 %)	21 (80 8 %)
Walking	24 (80 0 %)	21 (70 0 %)	26 (89 7 %)	22 (75 9 %)	22 (68.8 %)	19 (59 4 %)	21 (77 8 %)	18 (69.2 %)	17 (70.8 %)	19 (73 1 %)
Excitement	22 (73.3 %)	20 (66.7 %)	21 (72.4 %)	20 (69 0 %)	17 (53 1 %)	21 (65 6 %)	20 (74 1 %)	18 (69 2 %)	16 (66.7 %)	19 (73 1 %)
Chest pains in sleep	9 (30 0 %)	10 (33 3 %)	12 (41 4 %)	10 (34.5 %)	10 (31.3 %)	10 (31 3 %)	11 (40 7 %)	12 (46 2 %)	12 (50 0 %)	11 (42.3 %)
Other cause	—	5 (16.7 %)	1 (3 4 %)	2 (6.9 %)	2 (6 3 %)	2 (6.3 %)	2 (7 4 %)	4 (15 4 %)	3 (12.5 %)	2 (7 7 %)

* p < 0.05
** p < 0 01

Table XXIV Duration of angina pectoris attacks

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Minutes	98 (84.5 %)	108 (77.1 %)	86 (80.4 %)	94 (74.6 %)	82 (78.1 %)	83 (66.9 %)	84 (79.2 %)	79 (65.3 %)	84 (80.0 %)	88 (73.3 %)
1/2 hour	4 (3.5 %)	10 (7.1 %)	1 (0.9 %)	4 (3.2 %)	6 (5.7 %)	7 (5.6 %)	5 (4.7 %)	6 (5.0 %)	1 (1.0 %)	5 (4.2 %)
Hours	8 (6.9 %)	8 (5.7 %)	6 (5.6 %)	8 (6.3 %)	6 (5.7 %)	11 (8.9 %)	4 (3.7 %)	9 (7.4 %)	6 (5.7 %)	10 (8.3 %)
Min. + 1/2 h	1 (0.9 %)	4 (2.9 %)	3 (2.8 %)	2 (1.6 %)	1 (1.0 %)	6 (4.8 %)	1 (0.9 %)	4 (3.3 %)	3 (2.9 %)	3 (2.5 %)
Min + hs	5 (4.3 %)	7 (5.0 %)	9 (8.4 %)	15 (11.9 %)	8 (7.6 %)	11 (8.9 %)	7 (6.6 %)	18 (14.9 %)	8 (7.6 %)	10 (8.3 %)
1/2 h + hs	—	—	1 (0.9 %)	1 (0.8 %)	2 (1.9 %)	4 (3.2 %)	1 (0.9 %)	3 (2.5 %)	2 (1.9 %)	3 (2.5 %)
Min. + 1/2h + hs	—	3 (2.1 %)	1 (0.9 %)	2 (1.6 %)	—	2 (1.6 %)	2 (1.9 %)	2 (1.7 %)	1 (1.0 %)	1 (0.8 %)
Total	116 (100 %)	140 (100 %)	107 (100 %)	126 (100 %)	105 (100 %)	124 (100 %)	106 (100 %)	121 (100 %)	105 (100 %)	120 (100 %)

B Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		1 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Minutes	22 (73.3 %)	26 (86.7 %)	23 (79.3 %)	24 (82.8 %)	24 (88.9 %)	21 (77.8 %)	19 (70.4 %)	22 (84.6 %)	19 (79.2 %)	18 (69.2 %)
1/2 hour	4 (13.3 %)	1 (3.3 %)	3 (10.3 %)	1 (3.4 %)	—	1 (3.7 %)	3 (11.1 %)	—	1 (4.2 %)	—
Hours	1 (3.3 %)	1 (3.3 %)	1 (3.4 %)	1 (3.4 %)	1 (3.7 %)	1 (3.7 %)	2 (7.4 %)	1 (3.8 %)	2 (8.3 %)	2 (7.7 %)
Min. + 1/2 h	—	—	—	1 (3.4 %)	1 (3.7 %)	1 (3.7 %)	1 (2.7 %)	1 (3.8 %)	—	3 (11.5 %)
Min + hs	1 (3.3 %)	2 (6.7 %)	1 (3.4 %)	1 (3.4 %)	1 (3.7 %)	3 (11.1 %)	1 (3.7 %)	2 (7.7 %)	2 (8.3 %)	3 (11.5 %)
1/2 h + hs	1 (3.3 %)	—	—	1 (3.4 %)	—	—	1 (2.7 %)	—	—	—
Min. + 1/2h + hs	1 (3.3 %)	—	1 (3.4 %)	—	—	—	—	—	—	—
Total	30 (100 %)	30 (100 %)	29 (100 %)	29 (100 %)	27 (100 %)	27 (100 %)	27 (100 %)	26 (100 %)	24 (100 %)	26 (100 %)

Table XXV Grade of dyspnea

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No dyspnea	91 (63 6 %)	93 (56.0 %)	73 (54 9 %)	77 (51 0 %)	64 (50 4 %)	67 (46.2 %)	64 (52.0 %)	60 (42.6 %)	60 (49 6 %)	57 (41 0 %)
Grade I (dyspnea in jogging)	30 (21.0 %)	39 (23.5 %)	38 (28 6 %)	40 (26.5 %)	39 (30 7 %)	43 (29 7 %)	32 (26 0 %)	41 (29 1 %)	38 (31 4 %)	45 (32.4 %)
Grade II (dyspnea in walking)	21 (14 7 %)	30 (18.1 %)	21 (15 8 %)	30 (19 9 %)	22 (17 3 %)	25 (17.2 %)	25 (20.3 %)	30 (21.3 %)	21 (17 4 %)	30 (21 6 %)
Grade III (dyspnea in dressing)	1 (0 7 %)	3 (1.8 %)	1 (0 7 %)	4 (2.6 %)	2 (1 6 %)	8 (5.5 %)	2 (1 6 %)	8 (5 7 %)	1 (0 8 %)	6 (4.3 %)
Grade IV (dyspnea at rest)	—	1 (0 6 %)	—	—	—	2 (1 4 %)	—	2 (1 4 %)	1 (0 8 %)	1 (0 7 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No dyspnea	16 (43.2 %)	12 (35 2 %)	12 (34.3 %)	17 (50 0 %)	11 (34 4 %)	8 (25.0 %)	9 (29 0 %)	11 (37 9 %)	10 (35 7 %)	8 (27 6 %)
Grade I (dyspnea in jogging)	9 (24.3 %)	10 (29 4 %)	12 (34 3 %)	11 (32.4 %)	12 (37.5 %)	14 (43.8 %)	12 (38.7 %)	7 (24 1 %)	9 (32.1 %)	12 (41 4 %)
Grade II (dyspnea in walking)	11 (29 7 %)	11 (32.4 %)	11 (31 4 %)	6 (17 6 %)	9 (28 1 %)	9 (28 1 %)	10 (32.3 %)	11 (37 9 %)	9 (32.1 %)	7 (24 1 %)
Grade III (dyspnea in dressing)	1 (2.7 %)	1 (2.9 %)	—	—	—	1 (3 1 %)	—	—	—	2 (6 9 %)
Grade IV (dyspnea at rest)	—	—	—	—	—	—	—	—	—	—
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XXVI Physical activity in leisure time after MI

A. Men

	Initial checkup		3 month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Fully sedentary	114 (79.7 %)	112 (67.5 %)	50 (38.0 %)	71 (47.0 %)	42 (33.1 %)	60 (41.4 %)	38 (30.9 %)	61 (43.3 %)	31 (25.6 %)	54 (38.8 %)
Walking, cycling 4 hours/week	29 (20.3 %)	54 (32.5 %)*	79 (59.0 %)	78 (51.7 %)	82 (64.6 %)	83 (57.2 %)	82 (66.7 %)	77 (54.6 %)	86 (71.1 %)	81 (58.3 %)
Running, swimming etc 3 hours/week	—	—	4 (3.0 %)	2 (1.3 %)	3 (2.4 %)	2 (1.4 %)	2 (1.6 %)	3 (2.1 %)	4 (3.3 %)	4 (2.9 %)
Active training regularly several times/week	—	—	—	—	—	—	1 (0.8 %)	—	—	—
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Fully sedentary	36 (97.3 %)	33 (97.1 %)	26 (74.3 %)	25 (73.5 %)	23 (71.9 %)	24 (75.0 %)	21 (67.7 %)	22 (75.9 %)	16 (57.1 %)	23 (79.3 %)
Walking, cycling 4 hours/week	1 (2.7 %)	1 (2.9 %)	9 (25.7 %)	9 (26.5 %)	9 (28.1 %)	8 (25.0 %)	10 (32.3 %)	7 (24.1 %)	12 (42.9 %)	6 (20.7 %)
Running, swimming etc 3 hours/week	—	—	—	—	—	—	—	—	—	—
Active training regularly several times/week	—	—	—	—	—	—	—	—	—	—
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

* $p < 0.05$

Table XXV Grade of dyspnea

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No dyspnea	91 (63 6 %)	93 (56 0 %)	73 (54 9 %)	77 (51 0 %)	64 (50 4 %)	67 (46 2 %)	64 (52.0 %)	60 (42.6 %)	60 (49 6 %)	57 (41 0 %)
Grade I (dyspnea in jogging)	30 (21 0 %)	39 (23.5 %)	38 (28 6 %)	40 (26.5 %)	39 (30 7 %)	43 (29 7 %)	32 (26.0 %)	41 (29 1 %)	38 (31 4 %)	45 (32.4 %)
Grade II (dyspnea in alking)	21 (14 7 %)	30 (18 1 %)	21 (15.8 %)	30 (19 9 %)	22 (17.3 %)	25 (17.2 %)	25 (20.3 %)	30 (21.3 %)	21 (17 4 %)	30 (21 6 %)
Grade III (dyspnea in dressing)	1 (0 7 %)	3 (1 8 %)	1 (0 7 %)	4 (2 6 %)	2 (1 6 %)	8 (5.5 %)	2 (1 6 %)	8 (5 7 %)	1 (0.8 %)	6 (4.3 %)
Grade IV (dyspnea at rest)	—	1 (0 6 %)	—	—	—	2 (1 4 %)	—	2 (1 4 %)	1 (0 8 %)	1 (0 7 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
No dyspnea	16 (43.2 %)	12 (35 2 %)	12 (34 3 %)	17 (50 0 %)	11 (34 4 %)	8 (25.0 %)	9 (29.0 %)	11 (37 9 %)	10 (35 7 %)	8 (27 6 %)
Grade I (dyspnea in jogging)	9 (24.3 %)	10 (29 4 %)	12 (34.3 %)	11 (32.4 %)	12 (37.5 %)	14 (43 8 %)	12 (38.7 %)	7 (24 1 %)	9 (32.1 %)	12 (41 4 %)
Grade II (dyspnea in alking)	11 (29 7 %)	11 (32.4 %)	11 (31 4 %)	6 (17 6 %)	9 (28.1 %)	9 (28.1 %)	10 (32.3 %)	11 (37 9 %)	9 (32.1 %)	7 (24 1 %)
Grade III (dyspnea in dressing)	1 (2.7 %)	1 (2 9 %)	—	—	—	1 (3 1 %)	—	—	—	2 (6 9 %)
Grade IV (dyspnea at rest)	—	—	—	—	—	—	—	—	—	—
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XXVIII Claudication distance

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
< 100 m	3 (16.7 %)	12 (34.3 %)	2 (11.1 %)	9 (22.0 %)	1 (5.6 %)	8 (18.2 %)	1 (4.5 %)	10 (23.8 %)	1 (4.5 %)	9 (21.4 %)
100—299 m	9 (50.0 %)	12 (34.3 %)	6 (33.3 %)	9 (22.0 %)	8 (44.4 %)	19 (43.2 %)	7 (31.8 %)	13 (31.0 %)	8 (36.4 %)	12 (28.6 %)
300—499 m	1 (5.6 %)	5 (14.3 %)	1 (5.6 %)	11 (26.8 %)	2 (11.1 %)	5 (11.4 %)	1 (4.5 %)	5 (11.9 %)	3 (13.6 %)	9 (21.4 %)
500—999 m	4 (22.2 %)	3 (8.6 %)	6 (33.3 %)	7 (17.1 %)	4 (22.2 %)	7 (15.9 %)	6 (27.3 %)	9 (21.4 %)	5 (22.7 %)	9 (21.4 %)
> 1000 m	1 (5.6 %)	3 (8.6 %)	3 (16. %)	5 (12.2 %)	3 (16.7 %)	5 (11.4 %)	7 (31.8 %)	5 (11.9 %)	5 (22.7 %)	3 (7.1 %)
Total	18 (100 %)	35 (100 %)	18 (100 %)	41 (100 %)	18 (100 %)	44 (100 %)	22 (100 %)	42 (100 %)	22 (100 %)	42 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
< 100 m	—	2 (66.7 %)	1 (100 %)	2 (66.7 %)	1 (100 %)	2 (66.7 %)	—	2 (66.7 %)	—	3 (60.0 %)
100—299 m	1 (100 %)	1 (33.3 %)	—	1 (33.3 %)	—	—	1 (100 %)	—	—	1 (20.0 %)
300—499 m	—	—	—	—	—	—	—	—	—	—
500—999 m	—	—	—	—	—	1 (33.3 %)	—	1 (33.3 %)	—	1 (20.0 %)
> 1000 m	—	—	—	—	—	—	—	—	1 (100 %)	—
Total	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	5 (100 %)

Table XXVII Fatigue, claudication

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 143)	Control group (n = 166)	Training group (n = 133)	Control group (n = 151)	Training group (n = 127)	Control group (n = 145)	Training group (n = 123)	Control group (n = 141)	Training group (n = 121)	Control group (n = 139)
More fatigued than before MI	62 (43.4 %)	77 (46.4 %)	33 (24.6 %)	50 (33.1 %)	33 (26.0 %)	51 (35.2 %)	31 (25.2 %)	53 (37.6 %)*	28 (23.1 %)	44 (31.7 %)
Claudication	18 (12.6 %)	35 (21.1 %)	18 (13.4 %)	41 (27.2 %)**	18 (14.2 %)	44 (30.3 %)**	22 (17.9 %)	42 (29.8 %)*	22 (18.2 %)	42 (30.2 %)*

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 37)	Control group (n = 34)	Training group (n = 35)	Control group (n = 34)	Training group (n = 32)	Control group (n = 32)	Training group (n = 31)	Control group (n = 29)	Training group (n = 28)	Control group (n = 29)
More fatigued than before MI	22 (59.5 %)	27 (79.4 %)	13 (37.1 %)	15 (44.1 %)	15 (46.9 %)	16 (50.0 %)	10 (32.3 %)	9 (31.0 %)	10 (35.7 %)	11 (37.9 %)
Claudication	1 (2.7 %)	3 (8.8 %)	1 (2.9 %)	3 (8.8 %)	1 (3.1 %)	3 (9.4 %)	1 (3.2 %)	3 (10.3 %)	1 (3.6 %)	5 (17.2 %)

* $p < 0.05$
** $p < 0.01$

Table XXVIII Claudication distance

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
< 100 m	3 (16.7 %)	12 (34.3 %)	2 (11.1 %)	9 (22.0 %)	1 (5.6 %)	8 (18.2 %)	1 (4.5 %)	10 (23.8 %)	1 (4.5 %)	9 (21.4 %)
100—299 m	9 (50.0 %)	12 (34.3 %)	6 (33.3 %)	9 (22.0 %)	8 (44.4 %)	19 (43.2 %)	7 (31.8 %)	13 (31.0 %)	8 (36.4 %)	12 (28.6 %)
300—499 m	1 (5.6 %)	5 (14.3 %)	1 (5.6 %)	11 (26.8 %)	2 (11.1 %)	5 (11.4 %)	1 (4.5 %)	5 (11.9 %)	3 (13.6 %)	9 (21.4 %)
500—999 m	4 (22.2 %)	3 (8.6 %)	6 (33.3 %)	7 (17.1 %)	4 (22.2 %)	7 (15.9 %)	6 (27.3 %)	9 (21.4 %)	5 (22.7 %)	9 (21.4 %)
> 1000 m	1 (5.6 %)	3 (8.6 %)	3 (16.7 %)	5 (12.2 %)	3 (16.7 %)	5 (11.4 %)	7 (31.8 %)	5 (11.9 %)	5 (22.7 %)	3 (7.1 %)
Total	18 (100 %)	35 (100 %)	18 (100 %)	41 (100 %)	18 (100 %)	44 (100 %)	22 (100 %)	42 (100 %)	22 (100 %)	42 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
< 100 m	—	2 (66.7 %)	1 (100 %)	2 (66.7 %)	1 (100 %)	2 (66.7 %)	—	2 (66.7 %)	—	3 (60.0 %)
100—299 m	1 (100 %)	1 (33.3 %)	—	1 (33.3 %)	—	—	1 (100 %)	—	—	1 (20.0 %)
300—499 m	—	—	—	—	—	—	—	—	—	—
500—999 m	—	—	—	—	—	1 (33.3 %)	—	1 (33.3 %)	—	1 (20.0 %)
> 1000 m	—	—	—	—	—	—	—	—	1 (100 %)	—
Total	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	3 (100 %)	1 (100 %)	5 (100 %)

Table XXIX Smoking habits

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Non-smoker	12 (8.4 %)	15 (9.1 %)	11 (8.4 %)	15 (9.9 %)	11 (8.7 %)	15 (10.3 %)	11 (8.9 %)	15 (10.6 %)	11 (9.1 %)	15 (10.8 %)
Ex smoker	44 (30.8 %)	43 (25.9 %)	41 (30.8 %)	38 (25.2 %)	39 (30 %)	35 (24.1 %)	40 (32.5 %)	35 (24.8 %)	40 (33.1 %)	37 (26.6 %)
Gave up after present MI	39 (27.3 %)	48 (28.9 %)	29 (21.8 %)	37 (24.5 %)	28 (22.0 %)	37 (25.5 %)	24 (19.5 %)	34 (24.1 %)	24 (19.8 %)	30 (21.6 %)
Smoker	48 (33.6 %)	60 (36.1 %)	52 (39.0 %)	61 (40.4 %)	49 (38.6 %)	58 (40.0 %)	48 (39.0 %)	57 (40.4 %)	46 (38.0 %)	57 (41.0 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Non-smoker	23 (62.2 %)	22 (64.7 %)	21 (60.0 %)	22 (64.7 %)	18 (56.3 %)	20 (62.5 %)	18 (58.1 %)	17 (58.6 %)	17 (60.7 %)	18 (62.1 %)
Ex-smoker	1 (2.7 %)	4 (11.8 %)	1 (2.9 %)	4 (11.8 %)	1 (3.1 %)	3 (9.4 %)	1 (3.2 %)	2 (6.9 %)	1 (3.6 %)	2 (6.9 %)
Gave up after present MI	7 (18.9 %)	6 (17.7 %)	8 (22.9 %)	6 (17.6 %)	7 (21.9 %)	5 (15.6 %)	6 (19.4 %)	5 (17.2 %)	5 (17.9 %)	4 (13.8 %)
Smoker	6 (16.2 %)	2 (5.9 %)	5 (14.3 %)	2 (5.9 %)	6 (18.8 %)	4 (12.5 %)	6 (19.4 %)	5 (17.2 %)	5 (17.9 %)	5 (17.[illegible] %)
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XXX. Amount of tobacco consumed

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
1—14 g/day	35 (72.9 %)	51 (85.0 %)	37 (71.2 %)	51 (83.6 %)	37 (75.5 %)	45 (77.6 %)	34 (70.8 %)	45 (78.9 %)	34 (73.9 %)	42 (73.7 %)
15—24 — —	12 (25.0 %)	7 (11.7 %)	14 (26.9 %)	9 (14.8 %)	11 (22.4 %)	13 (22.4 %)	14 (29.2 %)	11 (19.3 %)	11 (23.9 %)	13 (22.8 %)
> 25 — —	1 (2.1 %)	2 (3.3 %)	1 (1.9 %)	1 (1.6 %)	1 (2.0 %)	—	—	1 (1.8 %)	1 (2.2 %)	2 (3.5 %)
Total	48 (100 %)	60 (100 %)	52 (100 %)	61 (100 %)	49 (100 %)	58 (100 %)	48 (100 %)	57 (100 %)	46 (100 %)	57 (100 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
1—14 g/day	6 (100 %)	2 (100 %)	5 (100 %)	2 (100 %)	6 (100 %)	4 (100 %)	6 (100 %)	5 (100 %)	4 (80.0 %)	5 (100 %)
15—24 — —	—	—	—	—	—	—	—	—	1 (20.0 %)	—
> 25 — —	—	—	—	—	—	—	—	—	—	—
Total	6 (100 %)	2 (100 %)	5 (100 %)	2 (100 %)	6 (100 %)	4 (100 %)	6 (100 %)	5 (100 %)	5 (100 %)	5 (100 %)

Table XXIX Smoking habits

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Non-smoker	12 (8.4 %)	15 (9 1 %)	11 (8.4 %)	15 (9 9 %)	11 (8.7 %)	15 (10.3 %)	11 (8 9 %)	15 (10 6 %)	11 (9 1 %)	15 (10 8 %)
Ex smoker	44 (30.8 %)	43 (25 9 %)	41 (30.8 %)	38 (25.2 %)	39 (30.7 %)	35 (24 1 %)	40 (32.5 %)	35 (24 8 %)	40 (33 1 %)	37 (26.6 %)
Gave up after present MI	39 (27.3 %)	48 (28.9 %)	29 (21 8 %)	37 (24.5 %)	28 (22.0 %)	37 (25.5 %)	24 (19.5 %)	34 (24 1 %)	24 (19.8 %)	30 (21 6 %)
Smoker	48 (33 6 %)	60 (36.1 %)	52 (39 0 %)	61 (40 4 %)	49 (38.6 %)	58 (40 0 %)	48 (39.0 %)	57 (40 4 %)	46 (38.0 %)	57 (41 0 %)
Total	143 (100 %)	166 (100 %)	133 (100 %)	151 (100 %)	127 (100 %)	145 (100 %)	123 (100 %)	141 (100 %)	121 (100 %)	139 (100 %)

B Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Non-smoker	23 (62.2 %)	22 (64 7 %)	21 (60 0 %)	22 (64 7 %)	18 (56.3 %)	20 (62.5 %)	18 (58 1 %)	17 (58 6 %)	17 (60 7 %)	18 (62.1 %)
Ex smoker	1 (2.7 %)	4 (11 8 %)	1 (2.9 %)	4 (11 8 %)	1 (3 1 %)	3 (9 4 %)	1 (3 2 %)	2 (6 9 %)	1 (3 6 %)	2 (6.9 %)
Gave up after present MI	7 (18.9 %)	6 (17 7 %)	8 (22.9 %)	6 (17 6 %)	7 (21 9 %)	5 (15 6 %)	6 (19 4 %)	5 (17 2 %)	5 (17 9 %)	4 (13 8 %)
Smoker	6 (16.2 %)	2 (5 9 %)	5 (14.3 %)	2 (5 9 %)	6 (18.8 %)	4 (12.5 %)	6 (19 4 %)	5 (17 2 %)	5 (17 9 %)	5 (17 2 %)
Total	37 (100 %)	34 (100 %)	35 (100 %)	34 (100 %)	32 (100 %)	32 (100 %)	31 (100 %)	29 (100 %)	28 (100 %)	29 (100 %)

Table XXXIII. Body weight; mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	72.7	—NS—	74.3	67.3	—NS—	65.7
	S.D.	10.4		10.1	11.0		11.0
	n	143		166	37		34
3-month checkup	Mean	73.8	—NS—	75.7	66.2	—NS—	65.8
	S.D.	9.8		10.0	10.3		10.3
	n	133		151	35		34
6-month checkup	Mean	73.3	—NS—	75.2	66.9	—NS—	65.8
	S.D.	10.0		10.2	9.9		9.6
	n	127		145	32		32
9-month checkup	Mean	73.2	—NS—	75.3	65.7	—NS—	65.5
	S.D.	9.9		10.4	10.1		9.5
		123		141	31		29
12 month checkup	Mean	73.2	—NS—	74.8	64.8	—NS—	66.2
	S.D.	9.8		10.4	9.8		9.4
		121		139	28		29

Control men: (paired t-test)
Initial checkup — 3-month checkup $p < 0.001$
— — — — — 6-month checkup $p < 0.01$
— — — — — 9-month checkup $p < 0.01$

Training women: (paired t-test)
Initial checkup — 9-month checkup $p < 0.01$
— — — — — 12 month checkup $p < 0.01$

Table XXXIV. Systolic blood pressure, mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	149.8	—NS—	151.4	172.7	—NS—	170.2
	S.D.	24.2		24.9	38.6		31.3
		143		166	37		34
3-month checkup	Mean	146.8	—NS—	147.9	165.7	—NS—	159.6
	S.D.	21.1		23.1	37.2		26.0
		133		151	35		34
6-month checkup	Mean	144.6	—NS—	147.5	170.8	—NS—	163.8
	S.D.	21.8		24.7	33.4		29.6
		127		145	32		32
9-month checkup	Mean	144.0	—NS—	146.9	167.6	—NS—	165.5
	S.D.	21.0		23.1	39.5		32.5
		123		141	31		29
12-month checkup	Mean	145.6	—NS—	150.9	157.4	—NS—	170.7
	S.D.	20.7		25.0	31.3		33.6
		121		139	28		29

Training men (paired t test)
Initial checkup — 3-month checkup $p < 0.05$
— — — — — 6-month checkup $p < 0.01$
— — — — — 9-month checkup $p < 0.01$
— — — — — 12 month checkup $p < 0.001$

Control men (paired t-test)
Initial checkup — 3-month checkup $p < 0.05$
— — — — — 6-month checkup $p < 0.05$
— — — — — 9 month checkup $p < 0.05$

Training women (paired t test)
Initial checkup — 12-month checkup $p < 0.05$

Control women (paired t test)
Initial checkup — 3-month checkup $p < 0.05$

Table XXXI Clinical findings, inspection, palpation and auscultation of the heart

A Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 143)	Control group (n = 166)	Training group (n = 133)	Control group (n = 151)	Training group (n = 127)	Control group (n = 145)	Training group (n = 123)	Control group (n = 141)	Training group (n = 121)	Control group (n = 139)
Inspection not normal	10 (7.0 %)	9 (5 4 %)	11 (8.2 %)	4 (2.6 %)	10 (7 9 %)	4 (2.8 %)	10 (8.1 %)	4 (2.8 %)	9 (7 4 %)	3 (2.2 %)
Aneurysm	3 (2.1 %)	2 (1.2 %)	3 (2.2 %)	1 (0 7 %)	3 (2.4 %)	1 (0 7 %)	3 (2.4 %)	1 (0 7 %)	2 (1 7 %)	1 (0 7 %)
A carotis slow	7 (4 9 %)	10 (6.0 %)	5 (3 7 %)	13 (8.6 %)	8 (6.3 %)	16 (11 0 %)	11 (8.9 %)	18 (12.8 %)	12 (9 9 %)	19 (13 7 %)
AS-murmur	1 (0 7 %)	5 (3 0 %)	1 (0 7 %)	5 (3.3 %)	1 (0 8 %)	5 (3 4 %)	1 (0.8 %)	5 (3.5 %)	1 (0.8 %)	3 (2.2 %)
AR murmur	1 (0 7 %)	1 (0 6 %)	—	1 (0 7 %)	—	1 (0 7 %)	—	1 (0 7 %)	—	—
MR murmur	29 (20.3 %)	29 (17.5 %)	30 (22.4 %)	23 (15.2 %)	33 (26 0 %)	27 (18.6 %)	31 (25 2 %)	28 (19 9 %)	35 (28.9 %)	24 (17.3 %)*
S1 diminished	32 (22.4 %)	17 (10 2 %)	23 (17 2 %)	15 (9 9 %)	23 (18 1 %)	15 (10.3 %)	20 (16.3 %)	11 (7 8 %)	18 (14 9 %)	14 (10 1 %)
S3 gallop	64 (44.8 %)	64 (38 6 %)	49 (36 6 %)	63 (41 7 %)	51 (40.2 %)	69 (47 6 %)	47 (38.2 %)	67 (47.5 %)	53 (43.8 %)	61 (43 9 %)
S4 gallop	109 (76.2 %)	138 (83 1 %)	107 (79 9 %)	129 (85 4 %)	107 (84.3 %)	132 (91 0 %)	106 (86 2 %)	131 (92.9 %)	112 (92.6 %)	131 (94 2 %)
RV pulsation	2 (1 4 %)	—	—	—	—	1 (0 7 %)	—	1 (0 7 %)	—	—
LV pulsation	23 (16.1 %)	21 (12.7 %)	23 (17.2 %)	23 (15 2 %)	21 (16.5 %)	23 (15 9 %)	22 (17 9 %)	19 (13.5 %)	24 (19 8 %)	17 (12.2 %)

p < 0 05

Table XXXII Clinical findings; Inspection, palpation and auscultation of the heart

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (n = 37)	Control group (n = 34)	Training group (n = 35)	Control group (n = 34)	Training group (n = 32)	Control group (n = 32)	Training group (n = 31)	Control group (n = 29)	Training group (n = 28)	Control group (n = 29)
Inspection not normal	4 (10.8 %)	2 (5 9 %)	4 (11 4 %)	2 (5 9 %)	6 (18.8 %)	2 (6.3 %)	4 (12.9 %)	2 (6 9 %)	5 (17 9 %)	3 (10.3 %)
Aneurysm	1 (2.7 %)	1 (2.9 %)	1 (2.9 %)	1 (2.9 %)	—	—	1 (3 2 %)	1 (3 4 %)	1 (3 6 %)	—
A.carotis slow	2 (5 4 %)	2 (5 9 %)	2 (5 7 %)	2 (5 9 %)	1 (3 1 %)	2 (6.3 %)	3 (9 7 %)	3 (10.3 %)	3 (10 7 %)	6 (20 7 %)
AS-murmur	5 (13.5 %)	1 (2.9 %)	5 (14.3 %)	1 (2.9 %)	4 (12.5 %)	1 (3 1 %)	4 (12.9 %)	2 (6.9 %)	4 (14.3 %)	2 (6.9 %)
AR murmur	1 (2 7 %)	—	1 (2.9 %)	—	1 (3 1 %)	—	1 (3 2 %)	—	1 (3 6 %)	—
MR murmur	7 (18.9 %)	14 (41 2 %)	10 (28.6 %)	15 (44 1 %)	11 (34 4 %)	12 (37.5 %)	10 (32.3 %)	11 (37 9 %)	10 (35 7 %)	13 (44.8 %)
S1 diminished	4 (10.8 %)	2 (5 9 %)	3 (8.6 %)	2 (5 9 %)	2 (6.3 %)	2 (6.3 %)	2 (6.5 %)	1 (3 4 %)	2 (7 1 %)	3 (10.3 %)
S3 gallop	19 (51 4 %)	14 (41 2 %)	17 (48.6 %)	17 (50 0 %)	15 (46 9 %)	18 (56.3 %)	18 (58.1 %)	18 (62 1 %)	15 (53 6 %)	19 (65.5 %)
S4 gallop	24 (64 9 %)	30 (88.2 %)*	22 (62.9 %)	30 (88.2 %)*	23 (71 9 %)	29 (90 6 %)	24 (77 4 %)	27 (93 1 %)	19 (67 9 %)	27 (93 1 %)
RV pulsation	—	1 (2 9 %)	—	1 (2.9 %)	—	1 (3 1 %)	—	—	1 (3 6 %)	—
LV pulsation	11 (29 7 %)	7 (20 6 %)	9 (25 7 %)	7 (20 6 %)	10 (31.3 %)	6 (18.8 %)	8 (25 8 %)	6 (20 7 %)	9 (32.1 %)	6 (20.7 %)

p < 0 05

AS = aortic stenosis
AR = aortic regurgitation
MR – mitral regurgitation
RV = right ventricle
LV = left ventricle

Table XXXIII Body weight; mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	72.7	—NS—	74.3	67.3	—NS—	65.7
	S.D	10.4		10.1	11.0		11.0
		143		166	37		34
3-month checkup	Mean	73.8	—NS—	75.7	66.2	—NS—	65.8
	S.D	9.8		10.0	10.3		10.3
		133		151	35		34
6-month checkup	Mean	73.3	—NS—	75.2	66.9	—NS—	65.8
	S.D	10.0		10.2	9.9		9.6
		127		145	32		32
9 month checkup	Mean	73.2	—NS—	75.3	65.7	—NS—	65.5
	S.D	9.9		10.4	10.1		9.5
		123		141	31		29
12-month checkup	Mean	73.2	—NS—	74.8	64.8	—NS—	66.2
	S.D	9.8		10.4	9.8		9.4
		121		139	28		29

Control men: (paired t-test)
Initial checkup — 3-month checkup $p < 0.001$
— — — — — — 6-month checkup $p < 0.01$
— — — — — — 9-month checkup $p < 0.01$

Training women (paired t-test)
Initial checkup — 9-month checkup $p < 0.01$
— — — — — 12-month checkup $p < 0.01$

Table XXXIV Systolic blood pressure; mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	149.8	—NS—	151.4	172.7	—NS—	170.2
	S.D	24.2		24.9	38.6		31.3
		143		166	37		34
3-month checkup	Mean	146.8	—NS—	147.9	165.7	—NS—	159.6
	S.D	21.1		23.1	37.2		26.0
	n	133		151	35		34
6-month checkup	Mean	144.6	—NS—	147.5	170.8	—NS—	163.8
	S.D	21.8		24.7	33.4		29.6
	n	127		145	32		32
9-month checkup	Mean	144.0	—NS—	146.9	167.6	—NS—	165.5
	S.D	21.0		23.1	39.5		32.5
		123		141	31		29
12-month checkup	Mean	145.6	—NS—	150.9	157.6	—NS—	170.7
	S.D	20.7		25.0	31.3		33.6
		121		139	28		29

Training men (paired t test)
Initial checkup — 3-month checkup $p < 0.05$
— — — — — — 6-month checkup $p < 0.01$
— — — — — — 9-month checkup $p < 0.01$
— — — — — — 12 month checkup $p < 0.001$

Control men. (paired t-test)
Initial checkup — 3-month checkup $p < 0.05$
— — — — — — 6-month checkup $p < 0.05$
— — — — — — 9 month checkup $p < 0.05$

Training women (paired t-test)
Initial checkup — 12 month checkup $p < 0.05$

Control women (paired t test)
Initial checkup — 3-month checkup $p < 0.05$

Table XXXV Diastolic blood pressure; mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	95 0	—NS—	94 7	101 4	—NS—	100 9
	S D	14.5		17 6	17 9		18.3
	n	143		166	37		34
3-month checkup	Mean	93.3	—NS—	92.8	98 1	—NS—	93 1
	S D	13 2		13 6	16.5		12.2
	n	133		151	35		34
6-month checkup	Mean	91 8	—NS—	94 0	99 2	—NS—	97.3
	S D	12.2		13.2	12.7		14 6
	n	127		145	32		32
9-month checkup	Mean	91.8	—NS—	92.9	99.3	—NS—	96.3
	S D	15.0		14 0	17 6		14 7
	n	123		141	31		29
12 month checkup	Mean	93 1	—NS—	95.3	93 4	—NS—	100.5
	S.D	12.9		16.3	13 9		15.0
	n	121		139	28		29

Training men. (paired t test)
Initial checkup — 6-month checkup p < 0 01
— — — — — 9-month checkup p < 0 05
— — — — — 12 month checkup p < 0 05

Control men (paired t test)
Initial checkup — 3-month checkup p < 0 01

Training women. (paired t test)
Initial checkup — 12 month checkup p < 0 01

Control women. (paired t test)
Initial checkup — 3-month checkup p < 0 05

Table XXXVI ECG findings

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group (= 143)	Control group (= 166)	Training group (= 133)	Control group (= 151)	Training group (= 127)	Control group (= 145)	Training group (= 123)	Control group (= 141)	Training group (= 121)	Control group (= 139)
QS finding	97 (67.8 %)	109 (65.7 %)	90 (67.2 %)	96 (63.6 %)	86 (67.7 %)	92 (63.4 %)	81 (65.9 %)	92 (65.2 %)	80 (66.1 %)	90 (64.7 %)
ST depression	129 (90.2 %)	132 (79.5 %)	106 (79.1 %)	120 (79.5 %)	101 (79.5 %)	109 (75.2 %)	93 (75.6 %)	99 (70.2 %)	91 (75.2 %)	100 (71.9 %)
Aneurysm	22 (15.4 %)	29 (17.5 %)	23 (17.2 %)	1 (13.9 %)	22 (17.3 %)	21 (14.5 %)	23 (18.7 %)	19 (13.5 %)	23 (19.0 %)	18 (12.9 %)
AV block gr I	4 (2.8 %)	3 (1.8 %)	4 (3.0 %)	2 (1.3 %)	4 (3.1 %)	2 (1.4 %)	3 (2.4 %)	2 (1.4 %)	2 (1.7 %)	3 (2.2 %)
LBBB	3 (2.1 %)	3 (1.8 %)	3 (2.2 %)	3 (2.0 %)	2 (1.6 %)	1 (0.7 %)	2 (1.6 %)	1 (0.7 %)	2 (1.7 %)	2 (1.4 %)
RBBB	4 (2.8 %)	4 (2.4 %)	4 (3.0 %)	4 (2.6 %)	4 (3.1 %)	4 (2.8 %)	4 (3.3 %)	2 (1.4 %)	4 (3.3 %)	—
Fibrillatio auricularis	2 (1.4 %)	4 (2.4 %)	2 (1.5 %)	5 (3.3 %)	2 (1.6 %)	3 (2.1 %)	2 (1.6 %)	3 (2.1 %)	2 (1.7 %)	3 (2.2 %)
Ectopic beats	9 (6.3 %)	17 (10.2 %)	5 (3.7 %)	10 (6.6 %)	3 (2.4 %)	6 (4.1 %)	9 (7.3 %)	9 (6.4 %)	10 (8.3 %)	6 (4.3 %)

Table XXXVII ECG-findings

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group (= 37)	Control group (= 34)	Training group (= 35)	Control group (= 34)	Training group (= 32)	Control group (= 32)	Training group (= 31)	Control group (= 29)	Training group (n = 28)	Control group (n = 29)
QS-finding	22 (59.5 %)	17 (50.0 %)	17 (48.6 %)	19 (55.9 %)	16 (50.0 %)	16 (50.0 %)	17 (54.8 %)	15 (51.7 %)	13 (46.4 %)	13 (44.8 %)
ST-depression	30 (81.1 %)	29 (85.3 %)	25 (71.4 %)	23 (67.6 %)	22 (68.8 %)	23 (71.9 %)	23 (74.2 %)	20 (69.0 %)	20 (71.4 %)	20 (69.0 %)
Aneurysm	3 (8.1 %)	3 (8.8 %)	2 (5.7 %)	3 (8.8 %)	2 (6.3 %)	3 (9.4 %)	2 (6.5 %)	3 (10.3 %)	2 (7.1 %)	3 (10.3 %)
AV block gr I	—	—	—	—	—	—	1 (3.2 %)	—	1 (3.6 %)	1 (3.4 %)
LBBB	2 (5.4 %)	—	2 (5.7 %)	—	1 (3.1 %)	—	1 (3.2 %)	—	1 (3.6 %)	—
RBBB	—	—	—	—	—	—	—	—	—	—
Fibrillatio auricularis	3 (8.1 %)	2 (5.9 %)	3 (8.6 %)	2 (5.9 %)	3 (9.4 %)	2 (6.3 %)	4 (12.9 %)	1 (3.4 %)	3 (10.7 %)	1 (3.4 %)
Ectopic beats	3 (8.1 %)	1 (2.9 %)	1 (2.9 %)	1 (2.9 %)	3 (9.4 %)	—	3 (9.7 %)	3 (10.3 %)	4 (14.3 %)	—

Table XXXV Diastolic blood pressure: mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	95 0	—NS—	94 7	101 4	—NS—	100 9
	S D	14.5		17 6	17.9		18.3
	n	143		166	37		34
3-month checkup	Mean	93.3	—NS—	92.8	98.1	—NS—	93 1
	S D	13.2		13 6	16.5		12.2
	n	133		151	35		34
6-month checkup	Mean	91 8	—NS—	94 0	99 2	—NS—	97.3
	S D	12.2		13 2	12.7		14 6
	n	127		145	32		32
9-month checkup	Mean	91 8	—NS—	92.9	99.3	—NS—	96.3
	S D	15 0		14 0	17 6		14 7
	n	123		141	31		29
12 month checkup	Mean	93 1	—NS—	95.3	93 4	—NS—	100.5
	S D	12.9		16.3	13 9		15 0
	n	121		139	28		29

Training men. (paired t test)
Initial checkup — 6-month checkup $p < 0\ 01$
— — — — — 9-month checkup $p < 0\ 05$
— — — — — 12 month checkup $p < 0\ 05$

Control men. (paired t test)
Initial checkup — 3-month checkup $p < 0\ 01$

Training women. (paired t test)
Initial checkup — 12 month checkup $p < 0.01$

Control women. (paired t test)
Initial checkup — 3-month checkup $p < 0.05$

Table XXXIX Roentgenologic findings

A. Men

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12-month checkup	
	Training group (= 143)	Control group (164)	Training group (= 133)	Control group (= 151)	Training group (= 127)	Control group (= 143)	Training group (= 123)	Control group (= 141)	Training group (= 121)	Control group (= 139)
Left atrial hypertrophy	20 (14.0 %)	17 (10.3 %)	21 (15.7 %)	14 (9.3 %)	19 (15.0 %)	15 (10.3 %)	16 (13.0 %)	10 (7.1 %)	14 (11.6 %)	7 (5.0 %)
Left ventricular hypertrophy	60 (42.0 %)	65 (39.4 %)	61 (45.5 %)	60 (39.7 %)	54 (42.5 %)	49 (33.8 %)	58 (47.2 %)	43 (30.5 %)**	54 (44.6 %)	46 (33.1 %)
Pulmonary vein congestion	37 (25.9 %)	40 (24.2 %)	33 (24.6 %)	35 (23.2 %)	23 (18.1 %)	31 (21.4 %)	24 (19.5 %)	33 (23.4 %)	21 (17.4 %)	32 (23.0 %)
Interstitial oedema	9 (6.3 %)	12 (7.3 %)	4 (3.0 %)	11 (7.3 %)	3 (2.4 %)	8 (5.5 %)	4 (3.3 %)	5 (3.5 %)	4 (3.3 %)	4 (2.9 %)

B. Women

	Initial checkup		3-month checkup		6-month checkup		9-month checkup		12 month checkup	
	Training group (= 37)	Control group (= 34)	Training group (= 35)	Control group (= 34)	Training group (= 32)	Control group (= 32)	Training group (= 31)	Control group (= 29)	Training group (= 28)	Control group (= 29)
Left atrial hypertrophy	9 (24.3 %)	6 (17.7 %)	9 (25.7 %)	6 (17.6 %)	7 (21.9 %)	5 (15.6 %)	9 (29.0 %)	2 (6.9 %)*	9 (32.1 %)	3 (10.3 %)
Left ventricular hypertrophy	19 (51.4 %)	8 (23.5 %)*	16 (45. %)	9 (26.5 %)	16 (50.0 %)	9 (28.1 %)	15 (48.4 %)	8 (27.6 %)	15 (53.6 %)	10 (34.5 %)
Pulmonary vein congestion	8 (21.6 %)	10 (29.4 %)	6 (17.1 %)	4 (11.8 %)	4 (12.5 %)	7 (21.9 %)	8 (25.8 %)	6 (20.7 %)	7 (25.0 %)	7 (24.1 %)
Interstitial oedema	5 (13.5 %)	5 (14.7 %)	5 (14.3 %)	4 (11.8 %)	5 (15.6 %)	2 (6.3 %)	6 (19.4 %)	1 (3.4 %)	5 (17.9 %)	1 (3.4 %)

* $p < 0.05$
** $p < 0.01$

Table XXXVIII Relative heart volume, cm /m mean values and standard deviations

		Training men		Control men	Training women		Control women
Initial checkup	Mean	495 8	—NS—	492.3	487 0	—NS—	451.2
	S D	118 6		115.2	135 8		99 1
	n	143		166	37		34
3-month checkup	Mean	495 6	—NS—	480 0	493 9	—NS—	441 1
	S D	117.2		104 7	149.2		96.2
	n	133		151	35		34
6-month checkup	Mean	497 7	— —	470.5	503 1	—NS—	443.3
	S D	123 0		94 4	148.9		103 4
	n	127		143	32		32
9-month checkup	Mean	503 1	— —	473 9	512.7	— —	449.5
	S D	128 7		110 1	148.9		81 9
	n	123		141	31		29
12 month checkup	Mean	505.5	—*—	473 9	529 8	— —	441 6
	S.D	132.0		93 4	178 8		95 6
	n	121		139	28		29

$p < 0.05$

Training men (paired t test)
Initial checkup — 9-month checkup $p < 0\ 05$
— — — — — 12 month checkup $p < 0\ 05$

Training women. (paired t test)
Initial checkup — 6-month checkup $p < 0\ 01$
— — — — — 9-month checkup $p < 0\ 001$
— — — — — 12 month checkup $p < 0\ 05$

Table XLII Ergometric tests: reason for the interruption of pedalling

A. Men

	1-month test		3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Angina pectoris	31 (43.1 %)	39 (48.8 %)	24 (46.2 %)	38 (54.3 %)	24 (58.5 %)	38 (61.3 %)	25 (61.0 %)	34 (45.9 %)	27 (73.0 %)	35 (46.1 %)
Dyspnea	23 (31.9 %)	23 (28.8 %)	18 (34.6 %)	23 (32.9 %)	10 (24.4 %)	14 (22.6 %)	13 (31.7 %)	28 (37.8 %)	7 (18.9 %)	28 (36.8 %)
Arrhythmia	3 (4.2 %)	—	—	—	—	1 (1.6 %)	—	—	—	—
Claudication	—	3 (3.8 %)	2 (3.8 %)	4 (5.7 %)	1 (2.4 %)	4 (6.5 %)	1 (2.4 %)	8 (10.8 %)	1 (2.7 %)	5 (6.6 %)
Leg fatigue	9 (12.5 %)	6 (7.5 %)	5 (9.6 %)	3 (4.3 %)	3 (7.3 %)	4 (6.5 %)	1 (2.4 %)	4 (5.4 %)	1 (2.7 %)	8 (10.5 %)
General fatigue	5 (6.9 %)	7 (8.8 %)	3 (5.8 %)	1 (1.4 %)	3 (7.3 %)	—	1 (2.4 %)	—	1 (2.7 %)	—
Dizziness	—	1 (1.3 %)	—	1 (1.4 %)	—	1 (1.6 %)	—	—	—	—
Heart rate exceeded 150 beats/min	1 (1.4 %)	1 (1.3 %)	—	—	—	—	—	—	—	—
Total	72 (100 %)	80 (100 %)	52 (100 %)	70 (100 %)	41 (100 %)	62 (100 %)	41 (100 %)	74 (100 %)	37 (100 %)	76 (100 %)

B. Women

	1-month test		3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Angina pectoris	7 (35.0 %)	8 (42.1 %)	4 (44.4 %)	10 (52.6 %)	4 (50.0 %)	9 (39.1 %)	3 (33.3 %)	7 (36.8 %)	2 (33.3 %)	8 (42.1 %)
Dyspnea	5 (25.0 %)	6 (31.6 %)	3 (33.3 %)	5 (26.3 %)	3 (37.5 %)	7 (30.4 %)	3 (33.3 %)	5 (26.3 %)	1 (16.7 %)	5 (26.3 %)
Arrhythmia	—	—	—	—	—	—	—	1 (5.3 %)	—	—
Claudication	—	—	—	—	—	2 (8.7 %)	—	2 (10.5 %)	—	—
Leg fatigue	3 (15.0 %)	2 (10.5 %)	1 (11.1 %)	2 (10.5 %)	1 (12.5 %)	3 (13.0 %)	2 (22.2 %)	2 (10.5 %)	2 (33.3 %)	4 (21.0 %)
General fatigue	3 (15.0 %)	2 (10.5 %)	1 (11.1 %)	2 (10.5 %)	—	2 (8.7 %)	1 (11.1 %)	1 (5.3 %)	1 (16.7 %)	2 (10.5 %)
Dizziness	—	—	—	—	—	—	—	1 (5.3 %)	—	—
Heart rate exceeded 150 beats/min	2 (10.0 %)	1 (5.3 %)	—	—	—	—	—	—	—	—
Total	20 (100 %)	19 (100 %)	9 (100 %)	19 (100 %)	8 (100 %)	23 (100 %)	9 (100 %)	19 (100 %)	6 (100 %)	19 (100 %)

Table XL. Metabolic variables; mean values and standard deviations

A Men

		3-month checkup			6-month checkup			9-month checkup			12 month checkup		
		Training		Control	Training		Control	Training		Control	Training		Control
Serum cholesterol mg/100 ml	Mean	278	—NS—	284	276	—NS—	285	281	—NS—	291	280	—NS—	290
	S D	49		53	47		51	51		51	50		56
	n	128		147	127		137	116		135	114		134
Serum triglycerides mg/100 ml	Mean	150	—NS—	174	151	—NS—	169	156	—NS—	192	155	—NS—	164
	S.D	81		144	102		130	87		453	83		106
	n	128		146	127		136	117		134	114		134
Serum urate mg/100 ml	*Mean*	*5.55*	*—NS—*	*5 94*	*5.50*	*—NS—*	*5.51*	*5.58*	*—NS—*	*5 61*	*5.58*	*—NS—*	*5.59*
	S.D	1.59		2.17	1 94		1.86	1 77		1.86	1 75		1 71
	n	127		146	127		136	116		135	113		134
Two-hour blood glucose value in oral glucose toler ance test mg/100 ml	Mean	98	—NS—	100	101	—NS—	101	100	—NS—	98	105	—NS—	99
	S D	36		35	31		36	36		29	39		37
	n	126		142	123		133	113		128	111		129

Training men. (paired t test)
Two-hour blood glucose value: 3-month checkup — 12-month checkup $p < 0 05$

Control men. (paired t test)
Serum urate: 3-month checkup — 6-month checkup $p < 0 05$
Serum cholesterol. 3-month checkup — 9-month checkup $p < 0 05$

Table XLI Metabolic variables, mean values and standard deviations

B. Women

		3-month checkup			*6-month checkup*			9-month checkup			12-month checkup		
		Training		Control	Training		Control	Training		Control	Training		Control
Serum cholesterol mg/100 ml	Mean	286	—NS—	304	289	—NS—	297	286	—NS—	301	300	—NS—	301
	S D	*60*		*54*	*66*		*61*	*51*		53	58		58
	n	32		33	32		31	30		27	28		25
Serum triglycerides mg/100 ml	Mean	149	—NS—	147	158	—NS—	149	167	—NS—	162	157	—NS—	156
	S D	41		64	95		77	99		90	78		80
	n	32		33	32		31	30		27	28		25
Serum urate mg/100 ml	Mean	5 25	—NS—	6 21	4 76	— —	5 97	5 21	—NS—	5 47	5 12	—NS—	5 41
	S D	2.12		2.23	1 49		2.28	1 44		2.73	1 69		1.81
	n	32		32	32		31	30		26	28		24
Two-hour blood glucose value in oral glucose toler ance test mg/100 ml	Mean	112	—NS—	120	116	—NS—	120	115	—NS—	116	104	—NS—	112
	S D	32		42	44		44	35		29	30		34
	n	31		29	32		29	30		26	28		23

$p < 0 05$

Table XLIV Physical working capacity 130, kpm min, mean values and standard deviations

B Women

1) All patients

	1-month test			3 month test			6-month test			9-month test			12-month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	328.4	—NS—	323.5	358.5	—NS—	343.4	405.6	—NS—	369.8	397.5	—NS—	401.4	358.7	—NS—	411.4
S D	144.0		104.9	179.5		114.4	188.3		128.2	160.5		110.1	154.3		132.8
	31		26	26		25	25		24	24		21	23		21

Training women (paired t test)

1 month test — 6 month test $p < 0.01$
1-month test — 9 month test $p < 0.05$

Control women (paired t test)

1-month test — 9-month test $p < 0.01$
1 month test — 12 month test $p < 0.001$

2) Patients who pedalled till their subjective maximum

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	329.0	—NS—	333.3	411.0	—NS—	415.0	442.5	—NS—	417.1	434.0	—NS—	508.6	385.0	—NS—	500.8
S.D	136.1		85.5	135.4		73.7	215.3		61.4	167.6		60.1	121.8		74.9
	5		9	5		7	4		7	3		7	5		6

Control women (paired t test)

1 month test — 9 month test $p < 0.001$
1 month test — 12 month test $p < 0.01$

T ble XLIII Physical working capacity 130 kpm/min, mean values and standard deviations

A Men

1) All patients

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	465 9	— —	526.2	532.7	—NS—	568 7	519 2	—** —	618.8	515 7	— —	624.5	533 6	—**—	619 1
S D	160.8		197 1	174.5		188.2	192.3		188 4	187 7		204 6	199 2		190.5
n	125		143	121		127	116		121	96		115	87		109

Training men. (paired t test)

1 month test — 3-month test $p < 0.001$
1 month test — 6-month test $p < 0.001$
1 month test — 9-month test $p < 0.001$
1 month test — 12 month test $p < 0.001$

Control men. (paired t test)

1 month test — 3-month test $p < 0.001$
1 month test — 6-month test $p < 0.001$
1 month test — 9-month test $p < 0.001$
1 month test — 12 month test $p < 0.001$

2) Patients bo pedalled till their subjective maximum

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	461 0	—NS—	539 0	512.8	—NS—	573 9	468 9	—**—	651.5	523 7	—NS—	637 2	562.6	— —	716.0
S D	150 0		240 1	160.8		227 4	153 9		225 1	157.5		269 1	186.2		256.9
	37		25	36		22	34		20	30		16	25		15

Training men. (paired t test)

1 month test — 3-month test $p < 0.01$
1 month test — 12 month test $p < 0.01$

Control men. (paired t test)

1 month test — 6-month test $p < 0.01$
1 month test — 9-month test $p < 0.05$
1 month test — 12 month test $p < 0.001$

$p < 0.05$
$p < 0.01$
** $p < 0.001$

Table XLIV Physical orking capacity 130 kpm/min, mean values and standard deviations

B Women

1) All patients

	1 month test			3 month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	328.4	—NS—	323.5	358.5	—NS—	343.4	405.6	—NS—	369.8	397.5	—NS—	401.4	358.7	—NS—	411.4
S D	144.0		104.9	179.5		114.4	188.5		128.2	160.6		110.1	154.3		132.8
	31		26	26		25	25		24	24		21	23		21

Training women. (paired t-test)

1 month test — 6-month test $p < 0.01$
1-month test — 9-month test $p < 0.05$

Control women (paired t-test)

1 month test — 9-month test $p < 0.01$
1 month test — 12 month test $p < 0.001$

2) Patients he pedalled till their subjective maximum

	1-month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	329.0	—NS—	338.3	411.0	—NS—	415.0	442.5	—NS—	417.1	434.0	—NS—	508.6	385.0	—NS—	500.8
S D	136.1		85.5	138.4		75.7	215.3		61.4	167.6		60.1	121.8		74.9
	5		9	5		7	4		7	5		7	5		6

Control omen. (paired t test)

1-month test — 9-month test $p < 0.001$
1-month test — 12 month test $p < 0.01$

Table XLIII Physical working capacity 130 kpm/min, mean values and standard deviations

A. Men

1) All patients

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	465 9	— —	526.2	532.7	—NS—	568 7	519.2	—** —	618 8	515 7	—***—	624.5	533 6	—**—	619 1
S D	160 8		197 1	174.5		188 2	192.3		188 4	187 7		204 6	199.2		190.5
n	125		143	121		127	116		121	96		115	87		109

Training men. (paired t test)

1 month test — 3-month test $p < 0\ 001$
1 month test — 6-month test $p < 0\ 001$
1 month test — 9-month test $p < 0\ 001$
1 month test — 12 month test $p < 0\ 001$

Control men. (paired t test)

1 month test — 3-month test $p < 0\ 001$
1 month test — 6-month test $p < 0.001$
1 month test — 9-month test $p < 0\ 001$
1 month test — 12 month test $p < 0\ 001$

2) Patients ho pedalled till their subjective maximum

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	461 0	—NS—	539 0	512 8	—NS—	573 9	468 9	— —	651.5	523 7	—NS—	637 2	562.6	— —	716 0
S.D	150.0		240 1	160 8		227 4	153 9		225 1	157.5		269 1	186.2		256 9
n	37		25	36		22	34		20	30		16	25		15

Training men. (paired t test)

1 month test — 3-month test $p < 0\ 01$
1 month test — 12 month test $p < 0\ 01$

Control men. (paired t test)

1 month test — 6-month test $p < 0\ 01$
1 month test — 9-month test $p < 0\ 05$
1 month test — 12 month test $p < 0\ 001$

$p < 0\ 05$
** $p < 0\ 01$
$p < 0.001$

Table XLVI. Physical working capacity improved more than 10 %

A. Men

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	67 (55.4 %)	62 (48.9 %)	63 (54.4 %)	78 (64.5 %)	48 (50.0 %)	66 (57.4 %)	46 (52.9 %)	64 (58.7 %)
No	54 (44.6 %)	65 (51.1 %)	53 (45.6 %)	43 (35.5 %)	48 (50.0 %)	49 (42.6 %)	41 (47.1 %)	45 (41.3 %)
Total	121 (100 %)	127 (100 %)	116 (100 %)	121 (100 %)	96 (100 %)	115 (100 %)	87 (100 %)	109 (100 %)

B. Women

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	11 (42.3 %)	13 (52.0 %)	19 (76.0 %)	13 (54.2 %)	14 (58.3 %)	11 (52.4 %)	10 (43.5 %)	12 (57.2 %)
No	15 (57.7 %)	12 (48.0 %)	6 (24.0 %)	11 (45.8 %)	10 (41.7 %)	10 (47.6 %)	13 (56.5 %)	9 (42.8 %)
Total	26 (100 %)	25 (100 %)	25 (100 %)	24 (100 %)	24 (100 %)	21 (100 %)	23 (100 %)	21 (100 %)

C. Men + women

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	78 (53.0 %)	75 (49.4 %)	82 (58.1 %)	91 (62.8 %)	62 (51.7 %)	77 (56.6 %)	56 (50.9 %)	76 (58.5 %)
No	69 (47.0 %)	77 (50.6 %)	59 (41.9 %)	54 (37.2 %)	58 (48.3 %)	59 (43.3 %)	54 (49.1 %)	54 (41.5 %)
Total	147 (100 %)	152 (100 %)	141 (100 %)	145 (100 %)	120 (100 %)	136 (100 %)	110 (100 %)	130 (100 %)

Table XLVII. Physical working capacity improved more than 20 %

A. Men

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	46 (38.0 %)	40 (31.5 %)	50 (43.1 %)	61 (50.4 %)	39 (40.6 %)	52 (45.2 %)	34 (39.1 %)	48 (44.0 %)
No	75 (62.0 %)	87 (68.5 %)	66 (56.9 %)	60 (49.6 %)	57 (59.4 %)	63 (54.8 %)	53 (60.9 %)	61 (56.0 %)
Total	121 (100 %)	127 (100 %)	116 (100 %)	121 (100 %)	96 (100 %)	115 (100 %)	87 (100 %)	109 (100 %)

B. Women

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	6 (23.1 %)	6 (24.0 %)	14 (56.0 %)	11 (45.8 %)	13 (54.2 %)	10 (47.6 %)	9 (39.1 %)	10 (47.6 %)
No	20 (76.9 %)	19 (76.0 %)	11 (44.0 %)	13 (54.2 %)	11 (45.8 %)	11 (52.4 %)	14 (60.9 %)	11 (52.4 %)
Total	26 (100 %)	25 (100 %)	25 (100 %)	24 (100 %)	24 (100 %)	21 (100 %)	23 (100 %)	21 (100 %)

C. Men + women

	3-month test		6-month test		9-month test		12-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Yes	52 (35.4 %)	46 (30.3 %)	64 (45.4 %)	72 (49.7 %)	52 (43.3 %)	62 (45.6 %)	43 (39.1 %)	58 (44.6 %)
No	95 (64.6 %)	106 (69.7 %)	77 (54.6 %)	73 (50.3 %)	68 (56.7 %)	74 (54.4 %)	67 (60.9 %)	72 (55.4 %)
Total	147 (100 %)	152 (100 %)	141 (100 %)	145 (100 %)	120 (100 %)	136 (100 %)	110 (100 %)	130 (100 %)

Table XLV Physical working capacity 130 kpm/min, mean values and standard deviations

C Men + women

1) All patients

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	438 6	—**—	495.0	501 9	—NS—	531 6	499 1	—** —	577 6	492.0	—***—	590 0	497 0	—***—	585 6
S D	166.5		199 6	187 1		196 6	195 9		202.1	187 9		209 0	203 0		197.5
n	156		169	147		152	141		145	120		136	110		130

Training men + women. (paired t test)

1 month test — 3-month test $p < 0\ 001$
1 month test — 6-month test $p < 0\ 001$
1 month test — 9-month test $p < 0\ 001$
1 month test — 12 month test $p < 0\ 001$

Control men + women. (paired t test)

1 month test — 3-month test $p < 0\ 001$
1 month test — 6-month test $p < 0\ 001$
1 month test — 9-month test $p < 0\ 001$
1 month test — 12 month test $p < 0\ 001$

2) Patents ho pedalled till their subjective maximum

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	445 2	—NS—	485 9	500 4	—NS—	535.5	465 9	— —	590 7	510 9	—NS—	598 0	533 0	— —	654.5
S D	153 1		228.0	160.3		211 6	158 0		221 0	159 6		232.4	187 8		239 8
n	42		34	41		29	38		27	35		23	30		21

Training men + omen (paired t test)

1 month test — 3-month test $p < 0\ 01$
1 month test — 9-month test $p < 0\ 05$
1 month test — 12 month test $p < 0\ 01$

Control men + women (paired t test)

1 month test — 3-month test $p < 0\ 05$
1 month test — 6-month test $p < 0\ 001$
1 month test — 9-month test $p < 0\ 01$
1 month test — 12 month test $p < 0\ 001$

$p < 0\ 05$
$p < 0\ 01$
*** $p < 0\ 001$

Table XLIX Ergometric tests, pulse rate before test and at the end of test, mean values and standard deviations

A. Men

		1 month test Training group	Control group	3-month test Training group	Control group	6-month test Training group	Control group	9-month test Training group	Control group	12-month test Training group	Control group
1) Pulse rate before test	Mean	86.2 —NS—	85.7	91.3 — **—	90.2	94.8 —***—	77.9	94.9 —***—	77.3	96.1 —***—	74.6
	S.D.	15.7	15.3	16.3	14.1	17.2	12.8	16.2	13.9	16.2	13.6
		132	163	110	147	112	140	103	138	95	138
2) Pulse rate at the end of test	Mean	128.2 —NS—	126.3	132.9 —**—	127.5	133.7 —***—	126.9	135.0 —***—	126.1	133.7 —**—	127.5
	S.D.	13.6	13.3	14.6	13.7	12.5	13.9	13.5	13.9	13.5	16.4
		132	163	110	147	112	140	103	137	95	138

B. Women

		1 month test Training group	Control group	3-month test Training group	Control group	6-month test Training group	Control group	9-month test Training group	Control group	12 month test Training group	Control group
1) Pulse rate before test	Mean	86.5 —NS—	85.6	92.3 — —	81.4	89.7 — —	80.2	93.5 —** —	78.4	99.0 —***—	76.6
	S.D.	12.8	15.8	19.5	17.0	17.3	15.4	16.3	10.9	15.9	12.5
		33	33	23	32	24	32	24	27	23	27
2) Pulse rate at the end of test	Mean	130.7 —NS—	132.5	138.1 — —	129.5	133.0 —NS—	126.4	133.2 — —	124.9	134.7 —NS—	137.0
	S.D.	14.7	14.4	13.2	13.8	11.5	14.4	10.3	13.4	11.0	16.4
		33	33	23	32	24	32	24	27	23	27

p < 0.05
p < 0.01
** p < 0.001

Table XLVIII Subjective maximum, kpm mean values and standard deviations

A. Men

	1 month test		3-month test		6-month test		9-month test		12 month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Mean	2544 —NS—	1953	3173 —NS—	2551	3244 —NS—	2705	3578 —*—	2503	3548 —NS—	2573
S D	1695	1385	1477	1868	1738	2169	1387	2149	1919	2573
n	42	36	40	30	36	28	33	28	28	30

Training men. (paired t test)

1 month test — 9-month test $p < 0.01$ 1 month test — 12 month test $p < 0.05$

B. Women

	1 month test		3-month test		6-month test		9-month test		13-month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Mean	1748 —NS—	1228	2026 — —	1062	1988 —NS—	1214	1552 —NS—	1408	1598 —NS—	1350
S D	721	634	709	678	686	645	746	757	818	865
n	5	12	5	13	4	11	5	9	5	9

C Men + omen

	1 month test		3-month test		6-month test		9-month test		12 month test	
	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
Mean	2459 — —	1771	3045 — —	2101	3118 — —	2284	3312 —**—	2237	3252 —NS—	2290
S.D	1633	1274	1453	1737	1701	1979	1485	1954	1922	2341
n	47	48	45	43	40	39	38	37	33	39

Training men + women. (paired t test)

1 month test — 3-month test $p < 0.01$ 1 month test — 6-month test $p < 0.05$
1 month test — 9-month test $p < 0.01$ 1 month test — 12 month test $p < 0.05$
Control men + omen (paired t test)
1 month test — 3-month test $p < 0.05$ 1 month test — 6-month test $p < 0.05$

$p < 0.05$
$p < 0.01$

Table XLIX Ergometric tests, pulse rate before test and at the end of test, mean values and standard deviations

A. Men

		1 month test Training group	Sig.	1 month test Control group	3-month test Training group	Sig.	3-month test Control group	6-month test Training group	Sig.	6-month test Control group	9-month test Training group	Sig.	9-month test Control group	12 month test Training group	Sig.	12 month test Control group
1) Pulse rate before test	Mean	86.3	—NS—	85.7	91.3	—***—	80.2	94.8	—***—	77.9	94.9	—***—	77.3	96.1	—***—	74.6
	S.D.	13.7		15.3	16.3		14.1	17.2		12.8	16.2		13.9	16.2		13.6
	(n)	132		163	110		147	112		140	103		138	95		138
2) Pulse rate at the end of test	Mean	128.2	—NS—	126.3	132.9	—**—	127.5	133.7	—**—	126.9	135.0	—***—	126.1	133.7	—**—	127.5
	S.D.	13.6		13.3	14.6		13.7	12.5		13.9	13.5		13.9	13.5		16.4
	(n)	132		163	110		147	112		140	103		137	95		138

B. Women

		1 month test Training group	Sig.	1 month test Control group	3-month test Training group	Sig.	3-month test Control group	6 month test Training group	Sig.	6 month test Control group	9-month test Training group	Sig.	9-month test Control group	12 month test Training group	Sig.	12 month test Control group
1) Pulse rate before test	Mean	86.5	—NS—	85.6	92.3	— —	81.4	89.7	—*—	80.2	93.5	—***—	78.4	99.0	—***—	76.6
	S.D.	12.8		15.8	19.5		17.0	17.3		15.4	16.3		10.9	15.9		12.5
	(n)	33		33	23		32	24		32	24		27	23		27
2) Pulse rate at the end of test	Mean	130.7	—NS—	132.5	138.1	— —	129.5	133.0	—NS—	126.4	133.2	— —	124.9	134.7	—NS—	127.0
	S.D.	14.7		14.4	13.2		13.8	11.5		14.4	10.3		13.4	11.0		16.4
	(n)	33		33	23		32	24		32	24		27	23		27

p < 0.05
p < 0.01
*** p < 0.001

Table L. Ergometric tests, systolic blood pressure (B.P) before test and at the end of test; mean values and standard deviations

A Men

		1 month test		3 month test		6-month test		9 month test		12 month test	
		Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
1) Systolic B P before test	Mean	151.2 —NS—	152.0	151 2 —NS—	150.4	151.3 —NS—	148 7	150 9 —NS—	147 0	148 7 —NS—	150 7
	S D	21.5	25 7	24 1	22.0	23 0	23 7	22.6	22.5	24 0	25 0
	n	132	163	110	147	112	140	103	138	95	138
2) Systolic B P at the end of test	Mean	179.8 —NS—	177 4	177 3 —NS—	179.5	176 4 —NS—	182.3	180.2 —NS—	179 9	173 7 —**—	184.3
	S D	28 8	31 1	29 0	30.5	30 1	30 9	28.8	29 9	28 6	30 0
	n	132	163	110	147	112	139	103	138	95	138

B Women

		1 month test		3-month test		6-month test		9-month test		12 month test	
		Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group	Training group	Control group
1) Systolic B.P before test	Mean	173 8 —NS—	178 3	171.3 —NS—	165 6	167 7 —NS—	165 6	169 8 —NS—	168.7	162.2 —NS—	173 0
	S D	28.7	31.5	29.3	30 8	26.5	31 7	34 0	32.6	29 6	33 7
	n	33	33	23	32	24	32	24	26	23	27
2) Systolic B P t the end of test	Mean	195 2 —NS—	191 8	199 6 —NS—	183.3	192.7 —NS—	191.5	192.7 —NS—	190 2	190 0 —NS—	200 9
	S D	39 9	37 9	36 4	31 9	31 2	35 4	35 4	32.1	37 7	34.5
	n	33	33	23	32	24	32	24	26	23	27

** $p < 0.01$

Table LI I[so]metric tests: diastolic blood pressure (B P) before test and at the end of test: mean values and standard deviations

A. Men

		1 month test Training group	1 month test Control group	3 month test Training group	3 month test Control group	6-month test Training group	6-month test Control group	9 month test Training group	9 month test Control group	12 month test Training group	12 month test Control group
1) Diastolic B P before test	Mean	96.3 —NS—	94.9	96.5 —NS—	93.8	95.5 —NS—	93.9	95.6 —NS—	93.0	96.8 —NS—	95.3
	S D	11.1	15.0	13.2	12.6	10.7	12.9	11.4	14.0	11.4	16.3
		132	163	110	147	112	140	103	138	95	138
2) Diastolic B P at the end of test	Mean	96.8 — —	90.1	96.4 —***—	87.2	92.5 — —	86.8	92.9 —NS—	90.1	91.8 —NS—	91.7
	S D	15.1	20.5	14.5	20.2	17.9	21.9	18.3	18.9	12.9	16.6
		132	163	110	147	112	139	103	138	95	138

B. Women

		1-month test Training group	1-month test Control group	3 month test Training group	3 month test Control group	6-month test Training group	6-month test Control group	9-month test Training group	9-month test Control group	12 month test Training group	12 month test Control group
1) Diastolic B P before test	Mean	98.6 —NS—	101.4	98.2 —NS—	95.2	98.3 —NS—	98.6	94.6 —NS—	96.3	96.5 —NS—	100.9
	S.D.	13.4	17.7	13.0	12.1	10.5	15.6	23.1	14.7	12.0	15.1
		33	33	23	32	24	32	24	26	23	27
2) Diastolic B P at the end of test	Mean	101.3 —NS—	97.6	93.0 —NS—	91.9	93.1 —NS—	94.7	95.2 —NS—	95.0	86.7 —NS—	97.2
	S D	18.7	22.3	25.8	12.5	13.4	16.5	11.9	17.7	22.1	16.4
		33	33	23	32	24	32	24	26	23	27

$p < 0.05$
*** $p < 0.001$

Table LII Ergometric tests, pulse rate — blood pressure product (10^3) at the end of test; mean values and standard deviations

A. Mean

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	22.9	—NS—	22.4	23 6	—NS—	22.9	23 6	—NS—	23 2	24 4	—*—	22.8	23 2	—NS—	23 6
S D	4.3		4 9	4 9		4.8	4 6		4 9	4 7		5.0	4 6		5.3
n	132		163	110		147	112		139	103		137	95		138

B Women

	1 month test			3-month test			6-month test			9-month test			12 month test		
	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group	Training group		Control group
Mean	25 7	—NS—	25 4	27.5	— —	23 9	25.5	—NS—	24 0	25 7	—NS—	23.8	25.5	—NS—	25 3
S D	6 0		5 7	5 2		4 4	4 0		4.3	5 2		4 7	4 9		4 1
n	33		33	23		32	24		32	24		26	23		27

$p < 0.05$
$p < 0.01$

Table LIII. Working capacity one year after MI

	Training men	Control men	Training women	Control women
Able to work after sick-leave	40 (28.0 %)	36 (21.7 %)	7 (18.9 %)	5 (14.7 %)
Tried to work, but had to retire	5 (3.5 %)	3 (1.8 %)	1 (2.7 %)	2 (5.9 %)
Sickness pension	92 (64.3 %)	113 (68.1 %)	28 (75.7 %)	22 (64.7 %)
Employment pension	1 (0.7 %)	—	—	1 (2.9 %)
Died	5 (3.5 %)	14 (8.4 %)	1 (2.7 %)	4 (11.8 %)
Total	143 (100 %)	166 (100 %)	37 (100 %)	34 (100 %)

Table LIV. Duration of sick-leave

	Training men	Control men	Training women	Control women
1— 3 months	5 (11.1 %)	3 (7.7 %)	1 (12.5 %)	3 (42.9 %)
4— 6 months	23 (51.1 %)	21 (53.9 %)	6 (75.0 %)	4 (57.1 %)
7—12 months	17 (37.8 %)	15 (38.5 %)	1 (12.5 %)	—
Total	45 (100 %)	39 (100 %)	8 (100 %)	7 (100 %)

Table LV. Residivous infarctions

	Training men (n = 143)	Control men (n = 166)	Training women (n = 37)	Control women (n = 34)
1 reinfarction	15 (10.5 %)	22 (13.3 %)	2 (5.4 %)	3 (8.8 %)
2 reinfarctions	1 (0.7 %)	1 (0.6 %)	1 (2.7 %)	3 (8.8 %)
3 reinfarctions	2 (1.4 %)	—	—	—
Total	18 (12.6 %)	23 (13.9 %)	3 (8.1 %)	6 (17.6 %)

Table LVI. Time (months) which elapsed before the first residivous infarction, mean values and standard deviations

	Training men	Control men	Training women	Control women
Mean	12.6	10.4	12.0	8.3
S.D.	9.8	9.0	16.5	6.0
n	18	23	3	6

Table LVII. Time lapse (months) between infarction and CHD-death

	Training men	Control men	Training women	Control women
Mean	19.4 — —	11.8	12.0	11.0
S.D.	12.6	9.0	1.4	3.2
n	16	22	2	6

$p < 0.05$

Table LVIII. Cause for death, autopsies, place of death, physical activity at moment of death, sudden deaths

	Training men	Control men	Training women	Control women
CHD death	16 (94.1 %)	22 (81.5 %)	2 (100 %)	6 (100 %)
Other cause for death	1 (5.9 %)	5 (18.5 %)	—	—
Autopsied	14 (82.4 %)	18 (66.7 %)	1 (50.0 %)	6 (100 %)
Died in hospital	11 (64.7 %)	15 (55.6 %)	1 (50.0 %)	4 (66.7 %)
Died at home	6 (35.3 %)	12 (44.4 %)	1 (50.0 %)	2 (33.3 %)
Died awake	14 (82.4 %)	24 (88.9 %)	2 (100 %)	5 (83.3 %)
Died in the nighttime	3 (17.6 %)	3 (11.1 %)	—	1 (16.7 %)
Died during physical activity	6 (35.3 %)	10 (37.0 %)	1 (50.0 %)	1 (16.7 %)
Died after training session	1 (5.9 %)	—	—	—
Died at rest	10 (58.8 %)	17 (63.0 %)	1 (50.0 %)	5 (83.3 %)
Sudden death	9 (52.9 %)	13 (48.2 %)	1 (50.0 %)	2 (33.3 %)

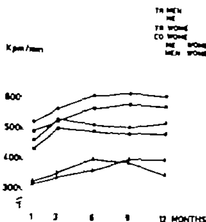


Fig 1 Physical working capacity 130 all patients

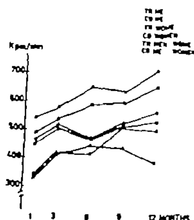


Fig 2. Physical working capacity 130; patients who pedalled till their subjective maximum.

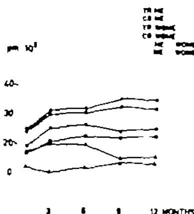


Fig 3. Subjective maximum

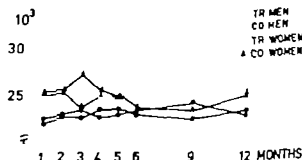


Fig 4 Pulse rate — blood pressure product at the end of test

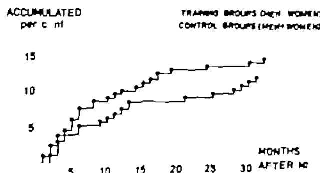


Fig 5. Accumulated per cent of reinfarctions in training groups and control groups



Fig 6 Accumulated per cent of CHD-deaths in training groups and control groups

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Long Term Non-Selective and Cardioselective Beta-Receptor Blockade in Hypertensive Patients

Effects on Circulatory Parameters, Catecholamines and Renin Activity under Basal Conditions and in Connection with Exercise and Hypoglycemia

By Bengt Göran Hansson

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This thesis is based on the following papers

I Hansson B G & Hökfelt B Long term treatment of moderate hypertension with penbutolol (Hoe 893d) I Effects on blood pressure pulse rate catecholamines in blood and urine plasma renin activity and urinary aldosterone under basal conditions and following exercise
Europ J clin Pharmacol 9 9-19 (1975)

II Hansson B -G & Hökfelt B Long term treatment of moderate hypertension with penbutolol (Hoe 893d) II Effect on the response of plasma catecholamines and plasma renin activity to insulin induced hypoglycemia
Europ J clin Pharmacol 9 241 251 (1976)

III Hansson B -G Dymling J -F , Hedeland H & Hulthén U L Long term treatment of moderate hypertension with the $beta_1$-receptor blocking agent metoprolol I Effect on maximal working capacity and on plasma catecholamines plasma renin activity urinary aldosterone blood pressure and pulse rate under basal conditions Submitted for publication

IV Hansson B -G Dymling J -F & Manhem P Long term treatment of moderate hypertension with the $beta_1$ receptor blocking agent metoprolol II Effect on the response of plasma catecholamines plasma renin activity blood pressure and pulse rate to submaximal work Submitted for publication

V Hansson B C & Hökfelt B Long term treatment of moderate hypertension with the $beta_1$ receptor blocking agent metoprolol III Effect on the response of plasma catecholamines and plasma renin activity to insulin-induced hypoglycemia Submitted for publication

In the text these papers will be referred to by their Roman numerals

CONTENTS

AIMS OF THE PRESENT STUDY

The following paper contains a summary and discussion of studies con cerning the influence of long term non selective and cardioselective beta receptor blockade in hypertension The studies particularly deal with the effect on blood pressure and pulse rate in relation to the production of catecholamines renin and aldosterone under basal and experimental conditions The patient material consisted of altogether 14 patients with moderate hypertension classified as essential in 13 of the cases The aims of the studies were

1) to investigate whether hypertension in these patients was directly related to the production of catecholamines and/or renin and aldosterone respectively

2) to analyze the possible relationship between sympathetic activity as measured by the determination of plasma catecholamines and the production of renin and aldosterone

3) to establish whether beta receptor blockade leads to major changes in the production of catecholamines and/or renin and aldosterone respectively which could explain the hypotensive effect of beta receptor blockade or which could lead to non desirable consequences of a circulatory and/or metabolic nature

4) to explore whether the two beta receptor blocking agents used exert effects not only via peripheral receptors but also via receptors located within the central nervous system

As a background to the presentation of the studies performed a short introduction will be given concerning catecholamines renin and mineralocorticoids

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INTRODUCTION

Various forms of cardiovascular and cerebrovascular diseases constitute the most common causes of death in the industrialized world One of the most important factors for the development of these diseases is hypertension (97 112 125 172 173) In spite of intense research etiology and pathogenesis still remain unsolved problems with respect both to essential hypertension and various forms of secondary hypertension During the last decades the search for pathogenetic mechanisms has to a great extent been concentrated to the question of the possible importance of the sympathetic nervous system and catecholamines the renin-angiotensin system and the mineralocorticoids

There is good evidence that drug-induced lowering of the blood pressure in hypertensives reduces mortality and probably also morbidity (112 125 172 173) Of fundamental importance for the accomplishment of such long term treatment has been the availability over the last 20 years of drugs effectively lowering blood pressure without major side effects Considerable efforts have been made to explore the pharmacological and pharmaco kinetic properties of these drugs It is noteworthy that the agents by far most commonly used in the treatment of hypertension are on one hand inhibitors of sympathetic activity at various levels and on the other hand those which influence the metabolism of electrolytes and water in the kidney and perhaps also at other sites These two types of agents thus have mechanisms of action which are closely linked to the three parameters emphasized above and which have been subjected to special investigations in the studies to be presented It seems possib le that the hypotensive properties of such agents used either alone or in combination actually reflect an action via mechanisms which are of etiological and/or pathogenetic importance

Amongst sympathetic inhibitors commonly used today the beta receptor blocking agents hold a prominent place Acceptable lowering of blood pressure can be obtained in a considerable number of hypertensives by medication with beta receptor blocking agents alone (60 76 144) and side effects usually are limited and comparatively mild (158) The

mechanism behind the antihypertensive effect of beta receptor blocking agents has not been clarified It has been attributed to inhibition of the sympathetic mediated effects on the heart (60 105 118 144 145) in hibition of the renin angiotensin system (27 28 169) re setting of the baro receptors (143 144) and to inhibition of the central nervous regulation of sympathetic activity (46 98 152)

Catecholamines

Under physiological conditions catecholamines are produced and released by adrenergic structures Principally these adrenergic structures can be divided into two categories namely neurons and chromaffin cells The neurons are located both within the central nervous system and peripherally as sympathetic postganglionic neurons The predominant part of the chromaffin cells is concentrated within the adrenal medulla

The synthesis of catecholamines starts from L tyrosine which via various enzymatic steps is converted to dopamine noradrenaline and adrenaline (Fig 1) While the catecholamines in the adrenal medulla are secreted into the blood in a hormone like manner both dopamine noradrenaline and adrenaline probably can function as transmittors in the nervous system Noradrenaline serves as a transmittor not only in peripheral sympathetic neurons but also within the central nervous system inclusive areas in the medulla oblongata and hypothalamus which are of importance for systemic blood pressure regulation (4) Dopamine for a long time was considered to be only a noradrenaline precursor but later on was found to play a role of its own as a transmittor both within certain part, of the central nervous system (140) and in peripheral neurons (65) Recent studies have demonstrated that also adrenaline probably can act as a transmittor within the central nervous system (85)

The sympathetic transmittor synthesized within the neuron is to a large extent stored in vesicles in the terminal part of the neuron On depolarization of the neuron the transmittor is released from these storage sites directly into the synaptic cleft where it interacts with a specific receptor at the postsynaptic membrane Termination of transmittor action is complex and seems to engage several mechanisms The

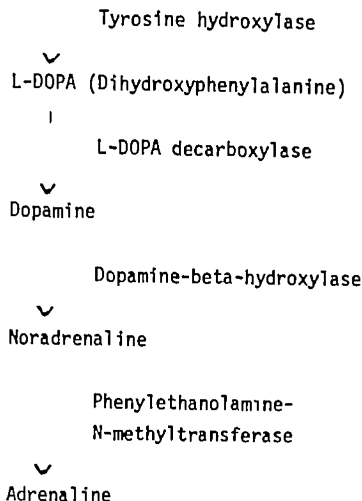


Fig 1

Intermediate stages in the formation of sympathetic amines

main part of the transmittor liberated is functionally inactivated by re-uptake into the sympathetic nerve terminal (174) where the trans mittor can either be newly stored in the vesicles or degraded by mono amine oxidase (MAO) present in the neuron Part of the transmittor re leased at the nerve terminals is transferred (by diffusion) into the systemic circulation and is rapidly catabolized either by 3 O-methyl ation under the influence of catechol O-methyltransferase (COMT) or deaminated via MAO This inactivation and degradation of catecholamines to a great extent occurs within the liver (73) and the metabolites are excreted either in the bile or in the urine A fraction of the active catecholamines circulating in the blood are excreted unchanged in the urine Whereas the noradrenaline circulating in the blood is mainly derived from peripheral sympathetic nerve endings circulating adren e

originates from the adrenal medulla At least to a certain extent the noradrenaline concentration in the blood reflects the activity of the peripheral sympathetic nervous system whereas the adrenaline content in a similar manner mirrors the secretory activity of the adrenal medulla

Several mechanisms exert an influence on the release of noradrenaline from the nerve terminals in connection with increased sympathetic activity The rate limiting enzyme in catecholamine synthesis L tyro sine hydroxylase is inhibited by catecholamines (155) Furthermore there exists in the nerve terminal an inhibitory alfa receptor located presynaptically (157) Alfa adrenergic stimulation for example by nor adrenaline exerts an inhibitory influence on the continued noradrenali ne release a mechanism constituting a short loop feed back mechanism Angiotensin II has been found to increase further the release of nor adrenaline normally following stimulation of the sympathetic neuron and this effect of angiotensin probably is due either to inhibition of noradrenaline re-uptake or to an increase in the amount of noradrena line released per nerve impulse (100 181)

The effects produced by liberated sympathetic neurotransmittors prin cipally are mediated via different kinds of adrenergic receptors These receptors have not been defined anatomically but should rather be look ed upon as functional unit. Ahlquist in 1948 published observations on basis of which he distinguished two types of adrenergic receptors (1) Investigating the effects of catecholamines on various organs he found that certain effector cells responded most promptly to stimulation with adrenaline and very little to isoprenaline and he classified this as an alfa receptor response Other cell receptors responded mainly to iso- prenaline and less to adrenaline and were classified as beta receptors Later on Lands likewise stimulating variou types of cells with diffe rent catecholamines further divided the beta receptors into two sub groups namely $beta_1$ for cardiac and $beta_2$ for bronchial and vascular receptors (106 107) Adrenaline as well as noradrenaline can stimulate both $beta_1$ and $beta_2$ receptors $Beta_1$ receptors are however most sen sitive to noradrenaline whereas $beta_2$ receptors respond most promptly to adrenaline (32 106 107) Examples of the localization and the bio logical effects mediated by the various types of adrenergic receptors

L-Tyrosine

Tyrosine hydroxylase



L-DOPA (Dihydroxyphenylalanine)

L-DOPA decarboxylase



Dopamine

Dopamine-beta-hydroxylase



Noradrenaline

Phenylethanolamine-
N-methyltransferase



Adrenaline

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are presented in Table 1 Recently evidence has been produced that alf receptors under experimental conditions can be transferred into beta receptors and vice versa (104) but the physiological importance of this finding is not clear Problems related to the identification of beta adrenergic receptors have recently been reviewed by Haber and Wrenn (72)

Table 1 Localization and action of some alpha- and beta-receptors

Organ	Receptor type	Action
Heart	$beta_1$	Increased rate and force of contraction
Resistance vessels	alpha	Constriction
	$beta_2$	Dilatation
Capitance vessels	alpha	Constriction
Bronchioli	$beta_2$	Relaxation
Adipose tissue	$beta_1$	Lipolysis
Liver	non-classified beta	Glycogenolysis

Stimulation of the beta receptor leads via activation of the enzyme adenylcyclase to increased production of cyclic AMP (8 160 161) con sidered to function as a second messenger with respect to the biologic al response evoked Alfa-receptor stimulation seems not to be connected with an increase in cyclic AMP but the possibility that cyclic GMP plays a role has been proposed (8)

The activity of the sympathetic neurons and the adrenal medulla is influenced by a number of endogenous and exogenous factors of physical and psychological nature (133) Of special interest with respect to the studies presented in the following is the well-known fact that sympath etic activity increases on standing and that this is accompanied by an increase in the blood catecholamine content (34 83 124 146) A further increase in sympathetic activity is seen following exercise and con comitant herewith plasma catecholamines increase in relation to the work

load applied (36 37 103 135) The secretion of adrenaline from the adrenals normally rather limited rises markedly in connection with hypoglycemia This effect is regulated via a central nervous mechanism and mediated by the splanchnic nerves (137) The close correlation between sympathetic activity and the blood content of catecholamines primarily noradrenaline is illustrated by the findings that patients with sympathetic insufficiency occurring in the Bradbury-Eggleston syndrome (19) and in tetraplegia as a result of damage to the spinal cord exhibit low catecholamine production both in supine and upright position and following hypoglycemia (80 117 124)

That overproduction of catecholamines can cause hypertension is illustrated in patients with phaechromocytoma Secretion of large amounts of catecholamines leads to vasoconstriction increased cardiac output and hypertension Whether the sympathetic nervous system plays a primary role for the establishment and/or maintenance of essential hypertension is not clear Patients with so called borderline hypertension a condition considered to precede established hypertension (113) often present clinical signs of increased sympathetic tone such as tachycardia elevated cardiac output and comparatively high levels of plasma renin activity (63 95 96) Studies in rats indicate that repeated psychological stress can result in hypertension (74)

Renin Angiotensin

The renal pressor ubstance renin discovered by Tigerstedt and Bergman in 1898 (162) attracted rather limited interest for a long time both amongst physiologists and clinicians When Goldblatt in 1934 (66) showed that experimentally produced constriction of the renal artery can lead to permanent hypertension renin again came into phocus but the interest gradually declined as the exact role of renin could not be defined The situation changed again following clarification of the nature of renin and its mode of action and during the two last decades a great number of studies have been presented dealing with the role of renin under physiological conditions and in connection with various types of hypertension

Renin is a proteolytic enzyme produced in the juxtaglomerular cells, located in the afferent arterioles in the renal glomeruli The chemical structure of renin is unknown It has a molecular weight of about 43 000 (148) In the general circulation renin interacts with renin substrate (angiotensinogen) an alfa-2-globulin synthesized within the liver to form the decapeptide angiotensin I Angiotensin I has a very low biological activity When circulating through the lungs and the kidneys angiotensin I is acted upon by converting enzyme which splits off the two terminal aminoacids to form an octapeptide angiotensin II which is a highly active vasoconstrictor and a potent stimulus to aldosterone secretion (108) Angiotensin II also stimulates sympathetic activity both at a central level and peripherally (see above) (100,181) In plasma renin has a half-life of about 15 20 min (150) whereas angio tensin II is inactivated very rapidly (14) by angiotensinases common ly distributed in the organism (99) It has been reported that a hep tapeptide produced from renin substrate angiotensin III can occur in peripheral human blood Angiotensin III can stimulate the secretion of aldosterone from adrenocortical tissue (68) but its physiological role is unknown

Several factors influence the renal release of renin In 1959 Tobian (163) proposed that the juxtaglomerular cells act as stretch receptors responding to changes either in intravascular afferent arteriolar pressure alterations in the transmural pressure of the afferent arte rioles at the site of the juxtaglomerular cells or wall tension at the same site Later studies have amply confirmed that changes in the mean renal artery blood pressure influence renin release (16 154) Thus constriction of the aorta above the renal arteries leading to a de crease in mean renal perfusion pressure is accompanied by an increase in renin release and the same holds true following general blood loss leading to hypotension (15) Supporting the stretch receptor hypo thesis is the finding that the renin release induced by major blood loss can be blocked by papaverine which prevents constriction of the arteriole (177) whereas denervation of the kidney has no influence on the renin release following reduction of mean renal arterial perfusion pressure (80 124)

Renin release is also influenced by the delivery of sodium to the macula densa and/or the sodium flux across the macula densa into the intersti tium near the juxtaglomerular apparatus (132)

Even though denervation of the kidney does not eliminate renin release following decreased mean renal arterial perfusion pressure there is good evidence that renal sympathetic nerve activity and circulating catecholamines exert a direct effect on renin secretion Thus direct stimulation of the renal nerves as well as stimulation of the medulla oblongata lead. to increased renin secretion (30 138 164 165)

The mechanism whereby circulating adrenaline and noradrenaline increase the release of renin is not definitely clarified There is good eviden ce both from in vivo and in vitro-studies that noradrenaline as such stimulates the liberation of renin (91 92 165 166 170 176) With re spect to adrenaline Johnsson et al (92) found that renin release in vivo can be inhibited by papaverine supporting the view that adrena line acts by changing the renal perfusion pressure On the other hand several studies in vitro have demonstrated release of renin from kid ney slices following incubation with adrenaline (91 170) The effect of dopamine on renin release has been studied but the results are dif- ficult to interpret because of a considerable influence of dopamine on renal circulation and sodium metabolism in the kidney (126)

Whether the effects of sympathetic nerve stimulation and circulating catecholamines on renin release are mediated via alfa or beta adren ergic mechanisms is still not fully clarified Both methoxamine a specific alfa receptor stimulating drug and isoprenaline a potent beta-receptor stimulating agent increase plasma renin activity (111) Most of the large number of studies within the field however support the view that the catecholamine induced renin release mainly is mediat ed via beta receptors (see below)

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In healthy individuals plasma renin activity increases on standing (39 136) A certain increase in renin activity can also occur in patients with sympathetic insufficiency (80) and tetraplegia (124) but in certain cases with sympathetic insufficiency complete absence of renin response to upright posture has been reported (69) Renin production also increases following major physical exercise and this seems to be related to the elevation of plasma catecholamines (36 59 103) and thus related to the work load applied (cfr above) Renin secretion is also closely related to sodium and water metabolism and aldosterone production restriction in intake and/or major losses of sodium and/or water leading to an increase in renin release and vice versa (23)

The role played by angiotensin II in blood pressure regulation under normal conditions is still an open question There is however evidence that angiotensin II is important for the maintenance of blood pressure in connection with sodium depletion Blockade of the action of angiotensin II by a competetive inhibitor in sodium depleted individuals leads to a pronounced decrease in blood pressure in upright position which is not the case in sodium replete subjects (63 71 149)

As angiotensin II is a very potent vasopressor agent its role in pathophysiological conditions has been subject to extensive studies Evidence that increased renin release can be of direct pathogenetic importance has been obtained in studies in certain forms of renovascular hypertension (167) and also in cases with a renin producing tumour (42) With regard to the importance of the renin angiotensin system for the establishment and maintenance of essential hypertension contradictory results have been obtained In some studies a correlation has been found between the suppression of plasma renin activity and the fall in blood pressure following certain types of treatment (27 169) but other studies have revealed no such correlation (21 77 78) It has been reported that expansion of the plasma volume in hypertensives leads to a rather minor decrease in renin production as compared to the reduction seen in normotensive individuals indicating that renin production in hypertensives is to some extent autonomous (101) If so this could represent circumstantial evidence for a pathogenetic role of the renin angiotensin system in essential hypertension

Reduction of plasma renin activity has been suggested to be crucial for treatment of hypertension The reason for this was that some investiga tors found a correlation between high levels of renin activity and risk for cardiova cular and/or cerebrovascular complications (25 26) Mo*t later studies exploring this question further have however failed to confirm such a relationship between plasma renin activity and hyperten sive complications (10 151)

During the last years there has been a trend to divide patients with essential hypertension in three groups based on their plasma renin values According to this classification about 25 per cent of the patients have low basal renin about 15 per cent have high renin values and the remaining 60 per cent normal renin activity (29 94) At the present time such a classification seems of limited interest because it has been found that also normal individuals of comparable age and sex demonstrate the same distribution of plasma renin activity (6)

Mineralocorticoids

The human adrenal cortex produces several steroids which influence the metabolism of sodium and potassium in the body The biologically most active of these mineralocorticoids produced under physiological con ditions is aldosterone This hormone causes sodium retention by stimu lating the reabsorption of sodium mainly in the distal tubules while at the same time increasing urinary potassium excretion In this way aldosterone can participate in the regulation of the plasma volume In situations of excessive aldosterone production such as primary aldo-steronism there is a tendency to hypernatremia and usually marked hypokalemia The production of aldosterone is influenced by angioten sin II and potassium which both increase aldosterone synthesis and re lease (18 41) Under ordinary conditions ACTH plays a conditioning role for aldosterone production but under certain circumstances ACTH can directly stimulate the secretion of aldosterone by the adrenal cortex (141) Potassium depletion causes inhibition of aldosterone production

There is good evidence that aldosterone can be of pathogenetic importan ce in hypertension as shown in patients with an aldosterone producing adrenal cortical tumour Conn s syndrome (41 178) Whether aldosterone

and/or other mineralocorticoids play a pathogenetic role in essential hypertension is not clear Most patients with essential hypertension produce and metabolize aldosterone in a normal manner (109) but a partly autonomous aldosterone production has been found in certain patients who failed to suppress aldosterone secretion following a sodium load (101)

Apart from aldosterone several other steroids with mineralocorticoid properties attract attention with respect to hypertension pathogenesis such as the aldosterone precursor 11-deoxy-corticosterone (DOC) and the DOC-metabolite 18 OH DOC Brown found that 6 out of 21 patients with hypertension and low plasma renin activity produced DOC in higher than normal amounts (24) Furthermore Melby reported increased urinary excretion of 18 OH DOC in 3 of 12 hypertensive patients likewise with low plasma renin activity (127) Liddle and co-workers (31) also claim that patients with essential hypertension and low plasma renin activity produce a salt retaining substance presumably a steroid of pathogenetic importance but so far attempts to define this substance chemically have not been successful

THE PRESENT STUDY

Beta receptor blocking agents used

Penbutolol (1 tert butylamino 3 (2-cyclo pentyl phenoxy)-propan 2-ol) is a non-selective beta receptor blocking agent without intrinsic sympatomimetic properties In animals it has been found to be ten times more potent than propranolol The half life in blood is not known but penbutolol has a comparatively long lasting effect on blood pressure and pulse rate (84)

The cardioselective beta receptor blocking agent metoprolol (($^{+}$) 1 (iso propylamino) 3 (p (2 metoxyethyl) phenoxy) 2-propanolol) likewise has no intrinsic sympatomimetic effects On a weight basis its $beta_1$ receptor blocking capacity is about equal to that of propranolol The half life in plasma has been estimated to 3-4 hours The pharmacology of metoprolol has been carefully studied by Ablad et al (183)

Patient material

In both studies patients were selected who under ambulatory conditions repeatedly showed moderate hypertension but no less than 170/110 in supine position after 30 minutes of rest Before acceptance for more detailed studies each patient was subjected to the following examina tion serum electrolytes serum creatinine urinary catecholamine excre tion/24 hours urinary protein X ray of the heart i v pyelography and radiorenogram and in some instances renal angiography Only if these investigations gave no evidence for the presence of secondary hyperten sion was a patient accepted for special studies

For the penbutolol studies four men and one woman were finally chosen and for the metoprolol studies nine men None of them had any other known disease except hypertension One of the patients (IV) in the pen butolol study had earlier been operated on because of renal artery stenosis but in spite of technically successful operation hypertension persisted and was therefore considered to be essential One patient (II) participating in the metoprolol studies was later found to have hereditary poly cystic renal disease in spite of presenting normal pyelographic and normal radiorenographic findings at the screening Initially

he also denied the presence of known hypertensive and/or renal disease within the family Clinical and laboratory findings in the two patient materials are summarized in Tables 2 and 3

Table 2 Clinical and laboratory findings in patients before treatment with penbutolol

Patient	Age	Supine bl pr (mmHg)	Eye ground KW	Cardiac [illegible] total/m²	U-aldosterone µg/24 hours	U-catecholamines µg/24 hours	Plasma renin activity ng/100 ml
I	45	163/107	I	470	11.6	36.9	68
II	46	147/104	II	470	17.3	26.7	63
III	25	181/125	0	330	31.9	17.1	730
IV	47	162/106	I	350	20.1	29.6	223
V	68	144/104	II	420	14.7	23.4	37
Normal range					3 15	10 80	50 200

Table 3 Clinical and laboratory findings in patients before treatment with metoprolol

Patient	Age	Supine bl.pr	Eye ground KW	Cardiac [illegible] total/m²	U-aldosterone µg/24 hours	U-catecholamines µg/24 hours	Plasma renin activity ng/100 1
I	47	185/103	I II	400	11.8	27.3	46
II	38	140/101	0	340	11.8	15.8	374
III	31	142/107	0	590		26.2	140
IV	44	164/111	0-I	500	10.8	33.4	132
V	38	130/98	0-I	495	13.2	29.3	290
VI	51	145/103	I	400	19.8	39.6	130
VII	45	148/105	II	380	27.6	30.3	47
VIII	50	152/93	0	300	18.9	37.9	198
IX	52	152/100	II	430	10.9	31.3	99

Oral medication with iron was given for one month after each period of special studies in the hospital The only additional medication given was penbutolol and metoprolol respectively

All patients were informed verbally about the intention and design of the studies (with the exception of medication with placebo) and gave their consent to participate

Study plan experimental procedures and analytical methods

The studies with penbutolol and metoprolol both had the same principal design Initially each patient was subjected to special studies (Table 4) without any kind of medication and the same kind of studies were repeated during medication with penbutolol and metoprolol respectively In the metoprolol studie identical investigations were also performed on placebo For the special studies the patients were hospitalized on a metabolic ward during 10 12 days During the ho pitalization periods the patients in the penbutolol study were given regular hospital diet whereas those in the metoprolol series received a diet containing 120 mEq of sodium and 95 mEq of potassium per day

Table 4 Special investigations performed before and during treatment with penbutolol and metoprolol respectively

Blood pressure and pulse rate in supine and upright position

Plasma catecholamines i supine and upright position

Plasma renin activity i supine and upright position

Basal 24 hour urinary excretion of aldosterone

Basal 24 hour urinary excretion of catecholamines (penbutolol studies only)

I fluence of subma imal work on blood pressure pulse rate plasma catecholamines and plasma reni activity

Influence of ins li -induced hypoglycemia on blood pressure pulse rate plasma catecholamines and plasma renin activity

Maximal working capacity

At the beginning of our investigations penbutolol had not been used clinically In order to obtain some information about the properties of the compound pilot studies were performed in two patients who initially were given a single dose of 2 mg per day after which the dosage was gradually increased In view of the information thus obtained the five patients were given a maintenance dosage of 20-30 mg twice a day for 3-8 months

he also denied the presence of known hypertensive and/or renal disease within the family Clinical and laboratory findings in the two patient materials are summarized in Tables 2 and 3

Table 2 Clinical and laboratory findings in patients before treatment with penbutolol

Patient	Age	Supine bl.pr (mmHg)	Eye ground KW	Cardiac size total/m²	U-aldosterone µg/24 hours	U-catecholamines µg/24 hours	Plasma renin activity ng/100 ml
I	[illegible]	163/107	I	470	13.6	16.9	68
II	46	147/104	II	470	17.3	18.7	65
III	25	181/125	0	330	31.5	17.1	730
IV	47	162/105	I	350	20.1	29.0	223
V	60	164/104	II	430	14.7	23.4	37
Normal range					3 15	10 50	50 200

Table 3 Clinical and laboratory findings in patients before treatment with metoprolol

Patient	Age	Supine bl pr	Eye ground KW	Cardiac size total/m²	U-aldosterone µg/24 hours	U-catecholamines µg/24 hours	Plasma renin activity ng/100 ml
I	47	158/103	I II	400	11.6	21.3	40
II	38	140/101	0	340	11.0	13.8	374
III	31	142/107	0	[illegible]	9.0	28.2	140
IV	44	154/111	0-I	500	14.0	33.8	122
V	38	138/98	0-I	455	13.2	25.1	300
VI	53	148/103	I	420	15.5	34.6	130
VII	48	140/101	II	390	27.5	30.3	477
VIII	40	182/113	0	350	16.9	37.0	190
IX	62	162/100	II	430	16.	31.3	99

Oral medication with iron was given for one month after each period of special studies in the hospital The only additional medication given was penbutolol and metoprolol respectively

All patients were informed verbally about the intention and design of the studies (with the exception of medication with placebo) and gave their consent to participate

Blood samples for the determination of plasma renin activity and plasma catecholamines were drawn repeatedly in supine position after over night bed rest and in upright posture after 30 minutes of peaceful walking around the ward The latter samples were used to obtain pre exercise control values to be compared with the values found in samples drawn immediately after completion of submaximal work In connection with the insulin test blood samples for the determination of glucose catecholamines and renin activity were drawn 10 min and immediately before the insulin injection and then after 30 45 60 and 120 minutes In all instances the blood samples were drawn from an antecubital vein All blood samples for the determination of plasma renin activity and catecholamines respectively were kept in a frozen state until analyz ed For each patient all samples collected durino one hospital stay were analyzed in one and the same series for catecholamines and plasma renin activity respectively

Plasma metoprolol concentrations were determined in blood samples drawn at 8 a m i e one hour after the last preceding medication Sample for such determinations were taken both under placebo and meto prolol

During each hospitalization period blood sampling caused a loss of about 400 ml of blood in each patient No replacement was given

Urine for the determination of aldosterone and catecholamines were collected for 24 hour periods without preservative and kept in a refrigerator For each patient all urine samples obtained during one hospital stay were as.ayed for aldosterone in the same single assay run

Analytical procedures u.ed for the determination of glucose cate cholamines renin activity aldosterone and metoprolol are presented in Table 5

In the metoprolol studies placebo tablets with a metoprolol like de sign were given half a tablet twice daily for four weeks Treatment with metoprolol was initiated by givning 25 mg three times daily and then gradually increased with intervals of two weeks until normotension or an acceptable decrease in blood pressure was obtained The main tenance dosage thus derived at amounted to 50-150 mg three times daily which was given 4-17 weeks The dose of 150 mg three times daily was not exceeded in any of the patients

Blood pressure was measured with a mercury manometer and registered on repeated occasions during hospitalization in supine position after 30 minutes of rest and immediately on standing, in connection with maxi mal and submaximal work and also during the studies including insulin induced hypoglycemia Simultaneously with blood pressure measurements pulse rate was registered by palpation

In the penbutolol series 4/5 patients and in the metoprolol studies all 9 patients were subjected to a standardized submaximal work test con sisting of exercise with the patient seated on a bicycle ergometer The load used was determined in advance in each patient individually without any kind of medication It was defined as the work load causing a steady state pulse rate of approximately 130/min This pre determined work load was applied for 6 minutes in all studies before and during medication In all instances the submaximal work test was performed at noon

In all patients maximal working capacity was determined Blood pressure and pulse rate were recorded before and during a work test consisting of exercise on a bicycle ergometer and with a step-wise increase in work load until the patient experienced symptoms or until the highest acceptable levels of blood pressure and/or pulse rate were reached

The effects of hypoglycemia were studied in all patients They were fasted and confined to bed from 10 p m on the day prior to the test Crystalline insulin (Vitrum) was injected intravenously at 8 a m in a dosage of 0 1 IU/kg body weight In control experiments 0 3 ml of physiological saline was given intravenously

RESULTS AND DISCUSSION

Catecholamines

Basal conditions Both groups of hypertensive patients studied before treatment had normal production of catecholamines in supine position and responded with a normal increase on standing (I III) The nor adrenaline concentration in peripheral blood varied considerably both intra and interindividually (Fig 2 and 3)

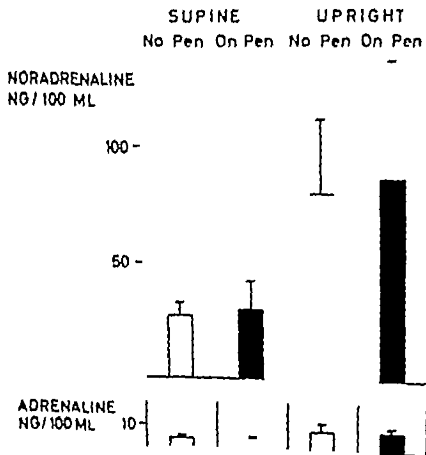


Fig 2 Plasma noradrenaline and adrenaline in supine and upright position before and during penbutolol (Pen)
Means ± SD are given

Table 5 Analytical procedures used

Blood glucose	Glucose oxidase method (122 130)
Plasma catecholamines	Double isotope derivative technique (48)
Urinary catecholamines	Fluorometry (58)
Plasma renin activity	Radioimmunoassay (70)
Urinary aldosterone	Double isotope derivative technique (102) (penbutolol studies)
	Radioimmunoassay (89) (metoprolol studies)
Plasma metoprolol	Gaschromatography (50)

Use of placebo

In the metoprolol studies placebo treatment was given to all patients during four weeks All patients had moderate hypertension without serious signs of organ complications At ambulatory examination following two weeks on placebo all patients were in good clinical condition and none showed signs of acceleration of the hypertensive disease

Statistical evaluation

Arithmetic means and standard deviations were calculated by conventional formulae Statistical significance of differences was assessed by the Student s t-test for paired data Significance was recorded for $p < 0\ 05$

RESULTS AND DISCUSSION

Catecholamines

Basal conditions   Both groups of hypertensive patients studied before treatment had normal production of catecholamines in supine position and responded with a normal increase on standing (I III) The nor adrenaline concentration in peripheral blood varied considerably both intra and interindividually (Fig 2 and 3)

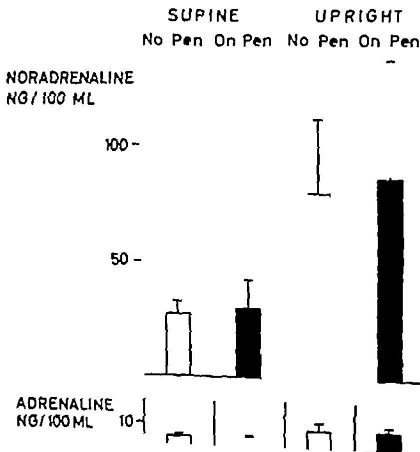


Fig 2   Plasma noradrenaline and adrenaline in supine and upright position before and during penbutolol (Pen)
Means ± SD are given

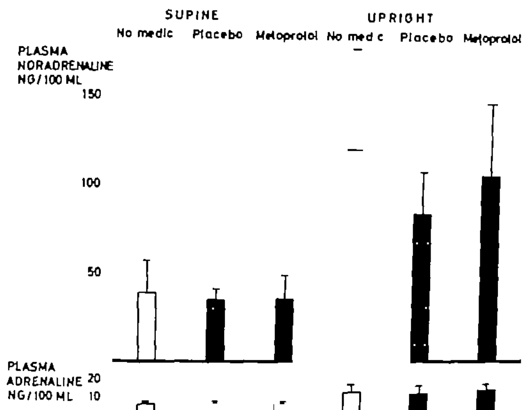


Fig 3 Plasma noradrenaline and adrenaline in supine and upright position before and during metoprolol Means ± SD are given

The catecholamine values found in these studies were of the same magnitude as those reported by Pedersen et al in a group of healthy subjects and also varied similarly (139) They differed however with respect to intraindividual variations from the values reported by de Champlain et al (34) who found only slight changes in plasma noradrenaline content from one day to the other after 20 min of rest In our studies plasma noradrenaline concentration increased 100 300 per cent on standing i e the same order of magnitude as reported in normotensive controls (43 124) The urinary catecholamine excretion measured over 24 hours and representing both rest in supine position and ordinary daily activities on the ward in up right position was similar to that found in normal subjects under basal conditions (Tables 2 and 3)

The values for catecholamines in blood and urine under basal condi tions in the two groups of patients were used to calculate the rela tionship between the catecholamine levels in plasma and the urinary

excretion of free noradrenaline and adrenaline For these calculations the plasma catecholamine values in supine and upright position were taken together and the mean for noradrenaline and adrenaline respectively was used A good correlation was found between the 24 hour urinary noradrenaline excretion and the plasma content of noradrenaline (r 0 72 p < 0 005) For adrenaline the correlation was less good (r 0 56 p < 0 0^c) These findings are noteworthy since there is rather good evidence that the urinary excretion of free noradrenaline amounts to only 1 5 3 3 per cent of the noradrenaline produced (54 67) For adrenaline the corresponding figures are 0 36 1 65 per cent (55)

The possibility that the sympathetic nervous system plays a role in essential hypertension has been intensly debated and extensively investigated by various means including determinations of catecholamines in plasma and urine under various conditions The catecholamine values found in our patients seem not to indicate any major abnormality in sympathetic function at least not in moderate hypertension With respect to the plasma levels of catecholamines in hypertensive patients our results are similar to those reported by Pedersen et al (139) Chodakowska et al (36) and Hanger et al (121) Elevated plasma levels of noradrenaline were however reported to occur in hypertensive patients by de Quatro et al (146) Engelman et al (49) Louis et al (114) and de Champlain et al (34) but at least in some of these studies hypertension seems to have been more severe It is of interest that Louis et al (114) reported a direct correlation between the level of plasma noradrenaline and the diastolic blood pressure in their patients In our studies diastolic pressure was rather uniform from one patient to the other within the two groups and not very high and no correlation was found between plasma catecholamine levels and diastolic blood pressure Pedersen et al (139) found that the plasma noradrenaline content increases with age a factor which seems not to have been taken into account in the studies by Louis et al In one study the urinary noradrenaline excretion in hypertensive patients was found to be inversely correlated to the diastolic blood pressure (12) but this might have been related to a concomitantly appearing decrease in glomerular filtration rate In several studies no statistically significant difference were found between the urinary excretion of free catecholamines in hyper

tensives as compared to normotensive healthy individuals (9 56 139) Indirect evidence for a role of the sympathetic nervous system in hypertension was presented by Mendlowitz et al (128) and Doyle et al (47) They found that patients with essential hypertension tend to have heightened vasoconstrictor response to infused noradrenaline

Neither penbutolol nor metoprolol significantly altered the plasma catecholamine content in supine or upright posture (Fig 2 and 3) (I III) Urinary catecholamine excretion was likewise the same before and during treatment with penbutolol under basal conditions (Fig 4)

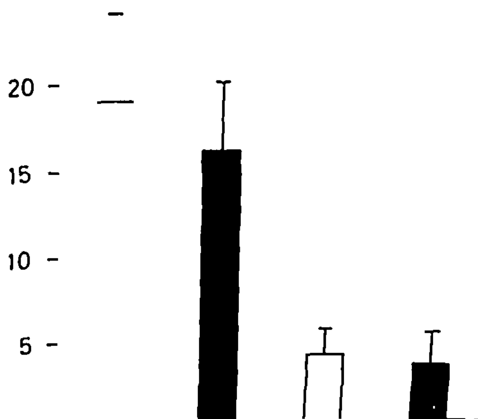


Fig 4 Urinary catecholamine excretion/24 hours under basal conditions before and during penbutolol (Pen) Means ± SD are given

For technical reasons measurements of urinary catecholamines could not be performed during treatment with metoprolol Only a few studies have been published concerning the effect of various beta receptor blocking agents on plasma catecholamine levels in supine and upright position Of interest with respect to our results are reports that neither pro pranolol oxprenolol nor pindolol induces changes in plasma catechol amines under basal conditions (3 22 87) Increased sympathetic activity is a prerequisite for adequate blood pressure regulation in upright position Since beta receptor blocking agents are considered to act mainly via competetive inhibition of sympathetically mediated effects one might expect a compensatory increase in the release of sympathetic transmittors to occur in connection with beta receptor blockade No such increase was found in our patients under basal conditions This probably reflects that the physical activity under these conditions represents only a relatively minor circulatory load Besides beta receptor blockade principally creates a situation where available cate cholamines more effectively stimulate the alfa receptors

Submaximal work In the penbutolol group the patients showed only a minor increase in plasma noradrenaline in response to work before medication (Fig 5) (I) whereas a definite increase was seen in the meto prolol group (Fig 6) (IV) In both groups plasma adrenaline varied considerably in connection with work before medication The divergent noradrenaline response observed in the two groups can probably be ex plained by the fact that the work load applied was heavier in the un treated metoprolol group in which the patients showed a lower pre exercise pulse rate without medication and accordingly had to work harder in order to reach 130 beats/min (submaximal work) As mention ed above the increase in plasma catecholamines during exercise is re lated to the work load applied Thus a relatively light work load in ducing an increase in pulse rate of less than 30 beats/min causes no measurable increase in circulating catecholamines (37) Actually there is good evidence that the increase in pulse frequency occurring under light work depends on a reduction of parasympathetic tone (37) A con siderable work load is *required before* a clear increase in plasma nor-adrenaline is seen and it is only after very hard work that an increase is seen also in plasma adrenaline (36 37 103) The increase in nor

tensives as compared to normotensive healthy individuals (9 56 139) Indirect evidence for a role of the sympathetic nervous system in hypertension was presented by Mendlowitz et al (128) and Doyle et al (47) They found that patients with essential hypertension tend to have heightened vasoconstrictor response to infused noradrenaline

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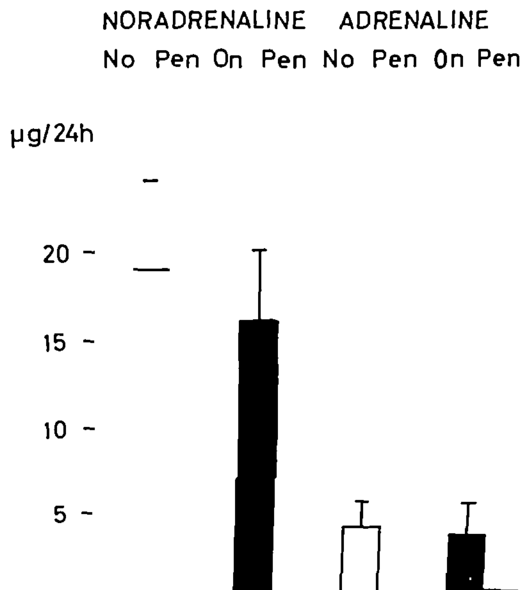


Fig 4 Urinary catecholamine excretion/24 hours under basal conditions before and during penbutolol (Pen) Means ± SD are given

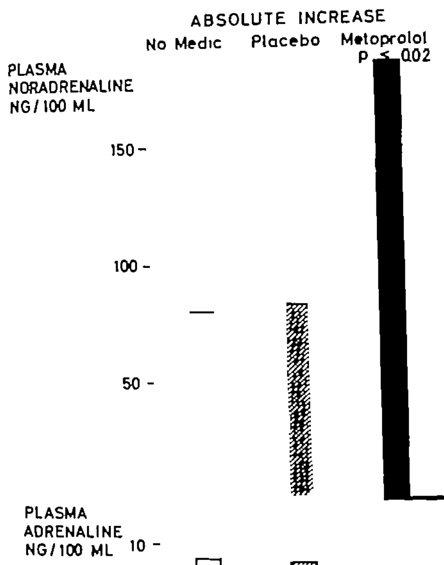


Fig 6 Absolute increase (means) in plasma catecholamines induced by submaximal work before and during metoprolol

During treatment with penbutolol the noradrenaline response to submaximal work increased markedly and there was also an increase in plasma adrenaline (Fig 5) Metoprolol on the other hand only caused a potentiation of the noradrenaline response whereas adrenaline did not change following submaximal work (Fig 6) The findings with penbutolol are in agreement with earlier reports concerning the effect

adrenaline in plasma following exercise seems to be greater in hyper tensives as compared to healthy controls (36) Our studies give no in formation concerning this question because no studies were performed in normotensive subjects

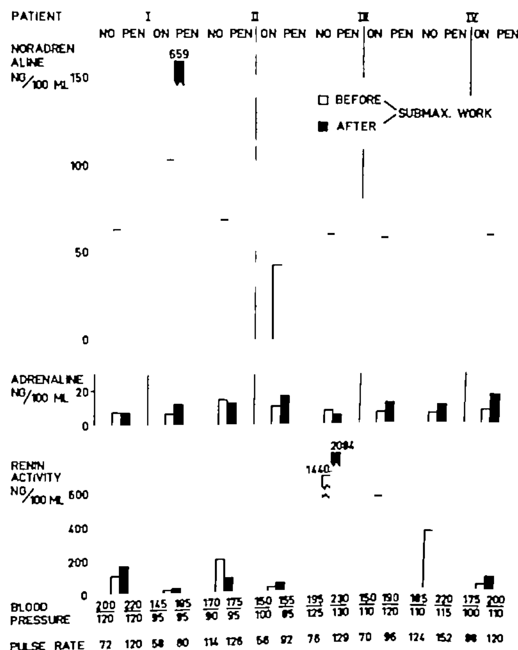


Fig 5 Plasma catecholamines and renin activity blood pressure and pulse rate before and immediately after submaximal work in patients I-IV without medication (NO PEN) and on treatment with penbutolol (ON PEN) 20-30 mg twice daily (Reproduced from Hansson & Hökfelt 1975 Euruop J clin Pharmacol by permission of the publishers)

The marked increase in plasma catecholamines occurring in connection with exercise during beta receptor blockade may also exert an influence on the production of various hormones and on various metabolic pro cesses which are regulated and/or modulated via various adrenergic receptors (13 33 62)

Hypoglycemia In both groups of patients the intravenous injection of a standardized dosage of insulin resulted in a similar degree of hypo glycemia before and during treatment with the respective beta receptor blocking agent In all cases blood glucose decreased approximately 50 per cent after 30-45 min (Fig 7 8 and 9) (II V) Before treatment hypoglycemia resulted in a marked increase in plasma adrenaline During treatment with penbutolol the adrenaline response to hypoglyce mia wa clearly reduced in four of the patients (Fig 7 and 8) whereas the results were difficult to interpret in the fifth patient It seems likely that reduction of the adrenaline response to hypoglycemia re- presents a typical effect of penbutolol In contrast to this a sig nificant increase in the adrenaline response to hypoglycemia was seen during metoprolol (Fig 9) Thus our investigations both with pen butolol and metoprolol illustrate that the adrenaline response to hypo glycemia can be modified by beta receptor blockade It is known that the adrenaline response to hypoglycemia is mediated via central nervous mechanisms Our studies seem to indicate that these mechanisms include a $beta_2$ receptor which can be blocked by a non-selective beta receptor blocking agent such as penbutolol but is unaffected by $beta_1$ receptor blockade with metoprolol Most non-selective beta receptor blocking agents have been found to penetrate into the central nervous system (123 159) and although there is no direct proof it seems likely that this applies also to penbutolol As demonstrated in animal experiments metoprolol also passes the blood brain barrier (17) The finding that intracerebroventricular injection of beta receptor blocking agents produces bradycardia and blood pre sure reduction (44 45 88 153 156) further supports the view that beta receptor blocking agents exert at least part of their antihypertensive effect via an influence on central nervous mechanisms

of the non-selective beta-receptor blocking agents propranolol pindolol and oxprenolol in normotensive subjects (22 87) The accelerated catecholamine response to work during medication with non-selective beta receptor blocking agents could be explained in several ways A comparatively high sympathetic activity might be required during such treatment to be able to perform a certain amount of physical work and the raised catecholamine levels in blood would then reflect a compensatory increase in sympathetic activity This hypothesis gains support by the finding that submaximal work during metoprolol accentuated only the noradrenaline response whereas no increase was found with respect to adrenaline These findings might indicate that the $beta_2$-receptors which are highly sensitive to adrenaline are not blocked under treatment with metoprolol (32 106 107) The increase in plasma catecholamines following exercise during treatment with beta receptor blocking agents could probably also depend on a reduced re-uptake of noradrenaline from the synaptic cleft under the influence of beta-receptor blocking agents resulting in an overflow of noradrenaline when sympathetic activity is accelerated (171) However in experimental studies there is also evidence for a decreased neuronal release of noradrenaline when sympathetic stimulation is performed under beta-receptor blockade (184) Another factor to be taken into account is that the catabolism of catecholamines to a great extent takes place in the liver (73) and that both exercise (119) and beta-receptor blockade reduce hepatic blood flow (38 134) which might alter the hepatic extraction and catabolism of catecholamines Furhtermore exercise causes a decrease in renal blood flow and glomerular filtration rate (35 86) which could also influence the plasma levels of catecholamines

Although the mechanism(s) cannot be fully explained our studies demonstrate that long term treatment of hypertensive patients with both non selective and cardioselective beta-receptor blocking agents leads to an acceleration of the plasma noradrenaline response to exercise It seems likely that these raised levels of noradrenaline further increase the alfa-receptor predominance already produced by beta receptor blockade per se which leads to a diminution of the skin blood flow and is responsible for the peripheral coldness often observed clinically (138)

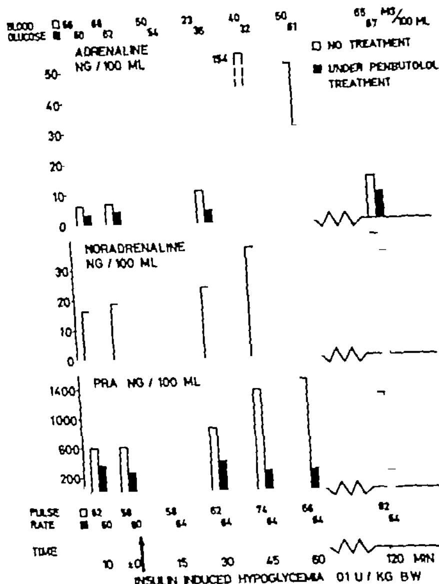


Fig 8 Blood glucose plasma adrenaline plasma noradrenaline plasma enin activity and pulse rate in connection with ins lin induced hypoglycemia before and during penbutolol in patient III

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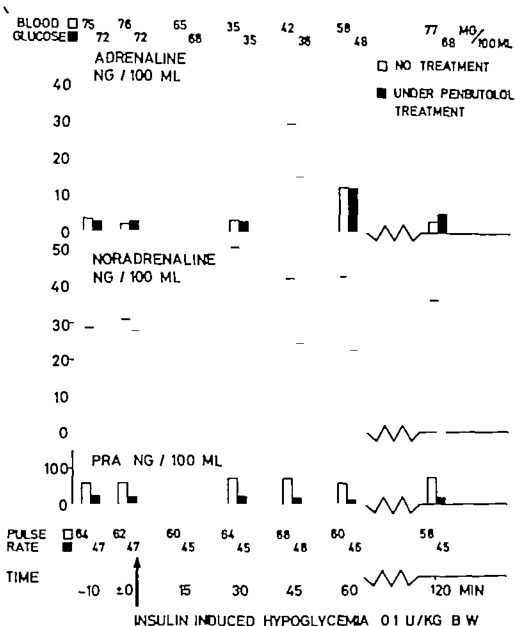


Fig 7 Blood glucose plasma adrenaline plasma noradrenaline plasma renin activity and pulse rate in connection with insulin induced hypoglycemia before and during penbutolol in patient I
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The increased adrenaline response to hypoglycemia observed during medication with metoprolol probably represents a compensatory mechanism to overcome the inhibitory effect of metoprolol on the $beta_1$ receptor mediated lipolysis (106 107 131)

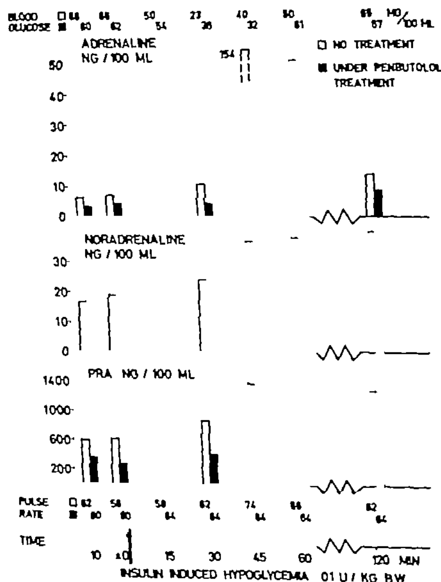


Fig 8 Blood glucose plasma adrenaline plasma noradrenaline plasma enin activity and pulse rate in connection with insulin induced hypoglycemia before and during penbutolol in patient 111
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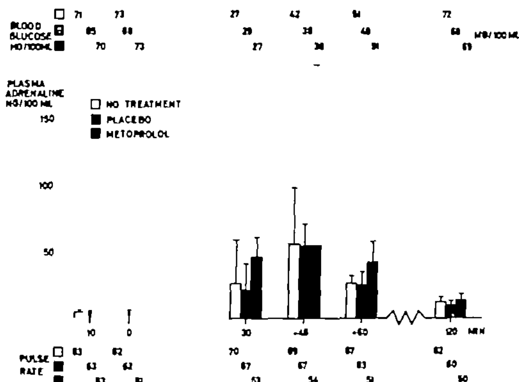


Fig 9 Blood glucose (means) plasma adrenaline (means ± SD) and pulse rate (means) in connection with insulin-induced hypoglycemia before and during metoprolol

The blood glucose lowering effect of insulin was not changed by treatment neither with penbutolol nor metoprolol This supports the view that these two beta-receptor blocking agents do not appreciably influence the sensitivity to insulin in man The return of blood glucose to normal following insulin-induced hypoglycemia showed the same pattern before and during penbutolol and metoprolol respectively This is of interest because at least in some studies beta-receptor blockade has been reported to decrease fasting blood glucose (13 62) but also to reduce the glucose induced increase in insulin production (33) These results have however been contradicted in other reports (82 147) It should also be recalled that studies in adrenalectomized patients demonstrate that an increase in adrenaline production is not a prerequisite for the normalization of blood glucose following insulin-induced hypoglycemia (57 64) The metabolic situation might however be markedly different under medication with for instance a beta-receptor blocking agent

Hypoglycemia was followed by a definite increase in plasma nor adrenaline in both groups of patients The response was of similar magnitude before and during medication with penbutolol and metoprolol respectively (Fig 7 8 and 10)

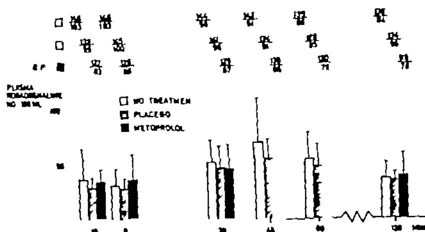


Fig 10 Blood pressure (means) and plasma noradrenaline (means $\pm$ SD) in connection with insulin-induced hypoglycemia before and during metoprolol

Renin-Angiotensin

Basal conditions In normal individuals plasma renin activity (PRA) increases in upright as compared to supine position Most patients with essential hypertension show the same pattern but the renin response to standing is decreased in patients with so called low renin hypertension (94) In our tudies all patients showed a definite increase in PRA in upright posture as compared to recumbency (Fig 11 and 12) (I III)

In the five patients treated with penbutolol PRA decreased in both supine and upright position (Fig 11) In fact penbutolol already in a single dosage of 2 mg suppressed PRA both at 4 and 24 hours after per oral administration Treatment with metoprolol was also accompanied by a significant decrease in supine and upright PRA (Fig 12) This reduction was closely correlated to the *concentration of* metoprolol in plasma (Fig 13)

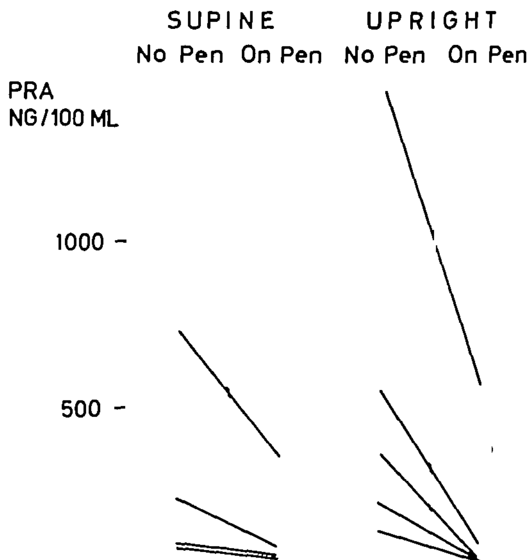


Fig 11 Mean plasma renin activity (PRA) in supine and up right position before and during penbutolol (Pen) in five patients

In agreement with several earlier studies concerning non selective beta receptor blocking agents our data with penbutolol illustrate that the release of renin can be modified by beta receptor blockade (20 75 77 129) Winer et al maintained that also alfa-receptor blockade reduces renin secretion (175) but this was not confirmed by Assaykeen et al (5) Our results demonstrating a definite decrease in PRA during metoprolol support the view that the release of renin at least partly is mediated via $beta_1$-receptors Similar findings have been reported previously (7 180 182) but there are also studies in which no effect on renin production was seen following $beta_1$-receptor blockade (2 52) al-

ternatively that the reduction was less effective with cardioselective as compared to non selective beta receptor blocking agents (90)

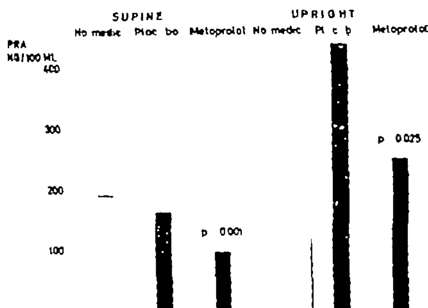


Fig 12 Mean plasma renin activity (PRA) in supine and upright position before and during metoprolol

The finding that the decrease in renin production during metoprolol was correlated to the plasma concentration of the drug could either indicate that $beta_1$ receptor blockade becomes more effective with increasing blood concentrations of metoprolol or that metoprolol in higher concentrations has both $beta_1$ and $beta_2$ receptor blocking properties There is some evidence that renin production can be modified via $beta_2$ receptors Thus the administration of salbutamol a $beta_2$ receptor stimulating compound is followed by increased PRA in vivo (91) The view that metoprolol in high concentrations can block also $beta_2$ receptors is supported by a report that bronchoconstriction can occur in asthmatic patients in connection with high plasma levels of metoprolol (93)

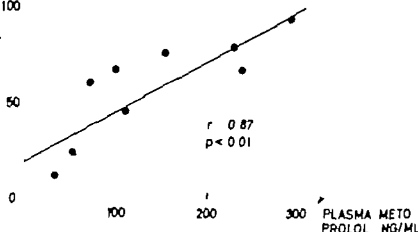


Fig 13 *Correlation between percentage supine PRA decrease following metoprolol and plasma metoprolol concen tration*

Taken together the two groups of patients before medication showed no correlation between the absolute values for plasma noradrenaline con centrations and PRA, neither in supine nor upright position (r = 0 21 p > 0 05 and r = -0 14 p > 0 05 respectively) However on standing there was a correlation between the percentage increase in plasma nor adrenaline and PRA (r = 0 69 p < 0 01) The finding that there is no correlation between absolute figures for plasma noradrenaline and PRA probably reflects the multifactorial regulation of renin production under basal conditions

Submaximal work Before medication submaximal work induced a definite increase in PRA in three of the four patients investigated in the pen butolol group (Fig 5) and in all patients in the metoprolol studies (Fig 14) (I IV) No definite explanation can be given for the absence of response in PRA following exercise in one of the patients but it might have been due to the fact that the increase in plasma noradrena line as measured simultaneously was relatively minor

Following submaximal work no correlation was found between the percen tage increase in plasma noradrenaline and PRA (r = 0 38 p > 0 05) In eleven of the thirteen patients the percentage increase in nor

adrenaline was more pronounced than the percentage increase in PRA The lack of correlation between the increase in plasma noradrenaline and PRA under these conditions is hardly surprising because the work test lasted only for six minutes and about 20-30 minutes are required for PRA to reach its maximum value following various stimuli such as upright posture and exercise (136) In contrast the response in plasma catecholamines is almost instantaneous (37) Because of the time lag between activation of the sympathetic system and renin release no conclusions can be drawn on basis of our results concerning the possible interrelationship between sympathetic activity and PRA

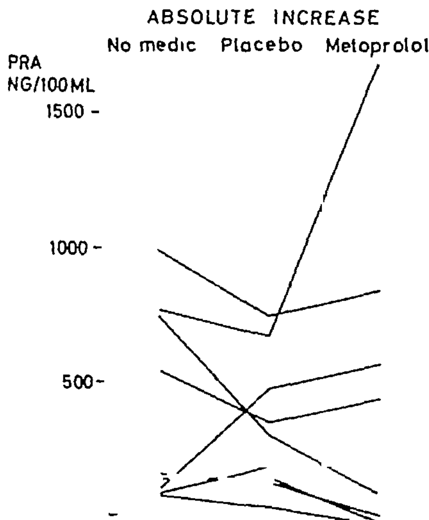


Fig 14 Absolute increase in plasma renin activity (PRA) induced by submaximal work before and during meto- p olol Individual alues are given

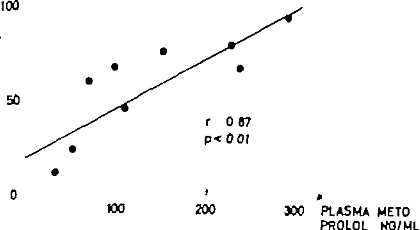


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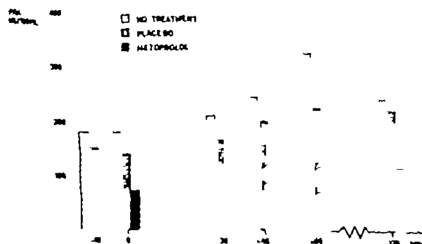


Fig 15 Plasma renin activity (PRA) in connection with insulin-induced hypoglycemia before and during metoprolol

The effect of non-selective beta receptor blockade on the PRA response to hypoglycemia found in our patients confirms earlier observations that non selective beta receptor blockade very effectively inhibits renin release under these conditions (5 116) So far no studies seem to have been published concerning the effect of cardioselective beta-receptor blocking agents on renin production following hypoglycemia

During penbutolol a marked reduction occurred in the renin response to exercise (Fig 5) The effect of metoprolol on the renin response to exercise varied however considerably In four patients the response was almost completely abolished whereas it was unaltered in five (Fig 14) This variation in renin response could not be explained on basis of differences in sympathetic activity measured as plasma noradrenaline content There was however a correlation between the concentration of metoprolol in blood and the inhibition of the plasma renin response The results might indicate an increased $beta_1$ receptor blockade at higher plasma concentrations of metoprolol Obviously there is also a possibility that the diverging results depend on renin release being modified also via $beta_2$-receptors (see above)

Hypoglycemia Before treatment the renin response to hypoglycemia in the penbutolol group seemed related to the basal levels Patients with low basal levels in supine position showed no response to hypoglycemia (Fig 7) whereas the others presented a definite response (Fig 8) (II) Similar results have been reported by Lowder et al (116) How ever in the metoprolol studies the level of basal PRA seemed not to predict whether the patient would respond to hypoglycemia with an in crease in PRA or not (Fig 15) (V) In those patients who before treatment responded with an increase in PRA during hypoglycemia this response was abolished during medication with penbutolol (Fig 8) During metoprolol three of the patients who responded with increased PRA before medication continued to do so whereas three others like wise with a definite renin response prior to treatment showed no in crease following hypoglycemia (Fig 15) In the remaining three pa tients no increase in PRA was seen following hypoglycemia neither be-fore nor during treatment with metoprolol

The increase in PRA following hypoglycemia has been attributed to the induced increase in circulating adrenaline (5 81 129 175) Before me dication all patients in the two groups even those who failed to in crease their PRA showed a pronounced increase in plasma catecholamines during hypoglycemia Variable renin responses to hypoglycemia have been reported previously by others (51) The mode of action of adrenaline on renin production seems to be rather complex as discussed in the in

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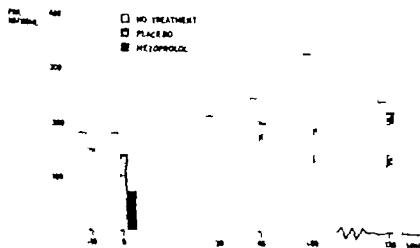


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centage reduction in supine PRA and also to the percentage reduction in the mean values of supine and upright PRA (r = 0 84 p < 0 01) (Fig 17) In spite of the fact that PRA in certain cases fell to very low levels the reduction in urinary aldosterone excretion was much less pronounced A decrease in urinary aldosterone has earlier been reported during treatment with propranolol (27) but also in these studies the lowering of PRA was much more pronounced than the reduction in urinary aldosterone This illustrates that mechanisms other than renin angiotensin are involved in the regulation of aldosterone production One such factor is serum potassium which however seemed not to have influenced aldosterone secretion in our studies because no changes were observed during treatment with any of the two beta receptor blocking agents

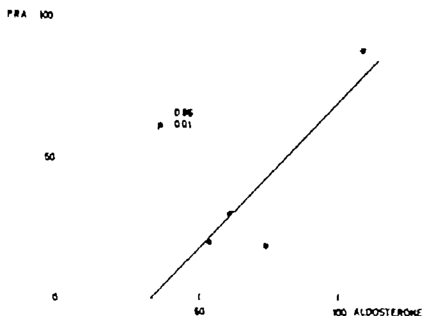


Fig 17 Supine plasma renin activity (PRA) and urinary aldosterone du ing metoprolol Values given are expressed in per cent of the values found be fore treatment

The variable effect seen in our studies supports the view that both beta$_1$- and beta$_2$-receptors can influence renin release The fact that metoprolol was very effective in some patients but rather ineffective in others, might reflect a variation from one patient to the other with respect to the number of beta$_1$- and beta$_2$-receptors in the juxtaglomerular apparatus alternatively a variation in the ratio between the two types of receptors

Mineralocorticoids

Three of the five patients in the penbutolol studies and two of the nine in the metoprolol series presented rather high urinary excretion of aldosterone prior to medication In three of these patients PRA was also comparatively high In the group as a whole treatment with penbutolol significantly lowered urinary aldosterone excretion (Fig 16) (I III)

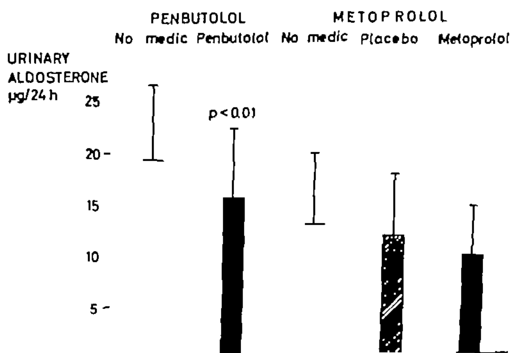


Fig 16 Urinary aldosterone (means ± SD) before and during penbutolol and metoprolol respectively

During treatment with metoprolol no significant decrease in urinary aldosterone was seen in the group as a whole (Fig 16) but a reduction occurred in six of eight patients In this latter study the percentage fall in aldosterone excretion was significantly correlated to the per

centage reduction in supine PRA and also to the percentage reduction in the mean values of supine and upright PRA (r 0 84 p < 0 01) (Fig 17) In spite of the fact that PRA in certain cases fell to very low levels the reduction in urinary aldosterone excretion was much less pronounced A decrease in urinary aldosterone has earlier been reported during treatment with propranolol (27) but also in these studies the lowering of PRA was much more pronounced than the reduction in urinary aldosterone This illustrates that mechanisms other than renin angiotensin are involved in the regulation of aldosterone production One such factor is serum potassium which however seemed not to have influenced aldosterone secretion in our studies because no changes were observed during treatment with any of the two beta receptor blocking agents

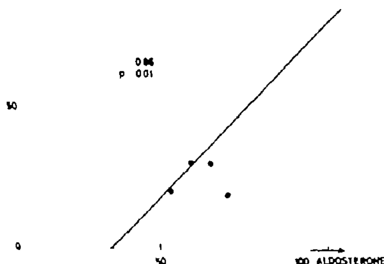


Fig 17 Supine plasma renin activity (PRA) and urinary aldosterone during metoprolol Values given are exp essed in per cent of the values found before treatment

Blood Pressure and Pulse Rate Working Capacity

Both penbutolol and metoprolol caused a reduction of blood pressure and pulse rate under basal conditions in supine as well as in upright position (Fig 18 and 19) and also in connection with submaximal (Fig 5 and 20) and maximal (Fig 21 and 22) work (I III IV)

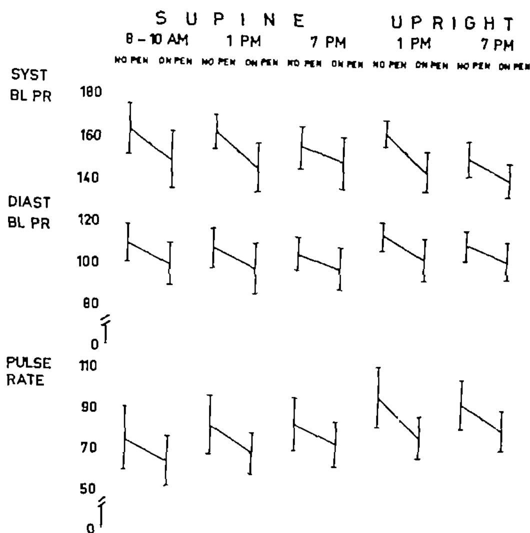


Fig 18 Blood pressure and pulse rate (mean values ± SD) before and during treatment with penbutolol (Reproduced from Hansson & Hökfelt 1975 Europ J clin Pharmacol by permission of the publishers)

These results agree with earlier reports concerning non selective (3 29 40 60 76 77 142 144) and cardioselective (2 29 52 79 182) beta receptor blocking agents With respect to the effect on blood pressure a considerable individual variation in dose dependency was noted both for penbutolol and metoprolol *The plasma concentration of the drug*

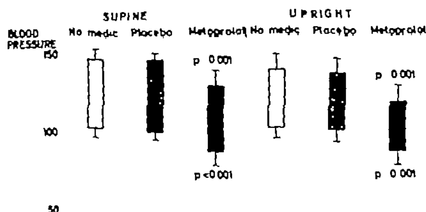


Fig 19 Blood pressure and pulse rate (means ± SD) in supine and upright position before and during metoprolol

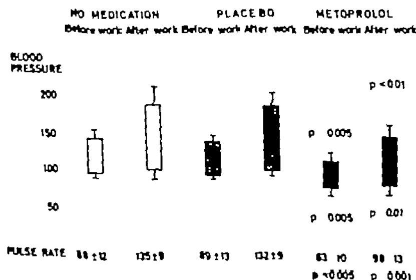


Fig 20 Blood pressure and pulse rate *before and after* submaximal work before and during *metoprolol*
Means ± SD are given

Blood Pressure and Pulse Rate Working Capacity

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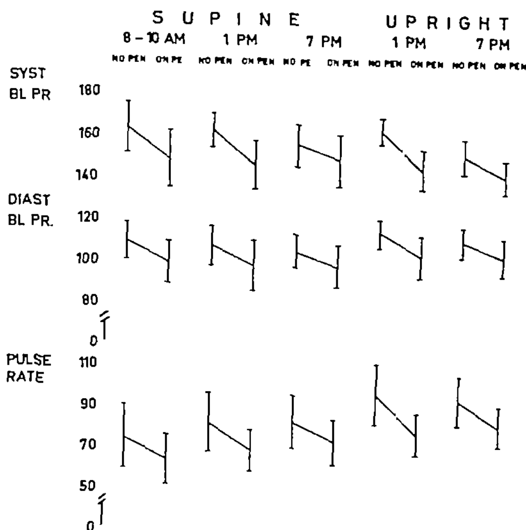


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possible that the antihypertensive properties of beta receptor blocking agents are due to both a central and a peripheral mode of action

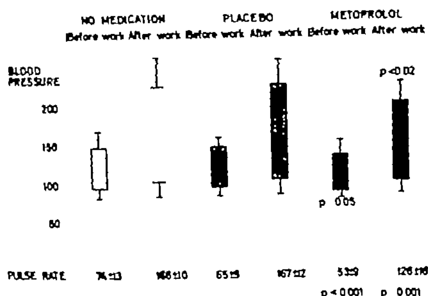


Fig 22 Blood pressure and pulse rate before and after maximal work before and during metoprolol
Means ± SD are given

The maximal working capacity estimated on a bicycle ergometer was not affected by treatment with penbutolol and metoprolol respectively Similar result have been reported during medication with propranolol (61 120) whereas no data seem to have been published with respect to cardioselective beta receptor blockade It should be noted that maximal working capacity remained unchanged during medication in spite of the fact that both beta receptor blocking agents reduced the exercise induced response in blood pressure and pulse rate

Side Effects

During treatment with penbutolol one patient who had no earlier history of asthma experienced light asthmatic symptoms (I) They were however so slight that the scheduled studies could be continued as planned After completion of the investigations the patient was transferred to another kind of antihypertensive medication It is well known

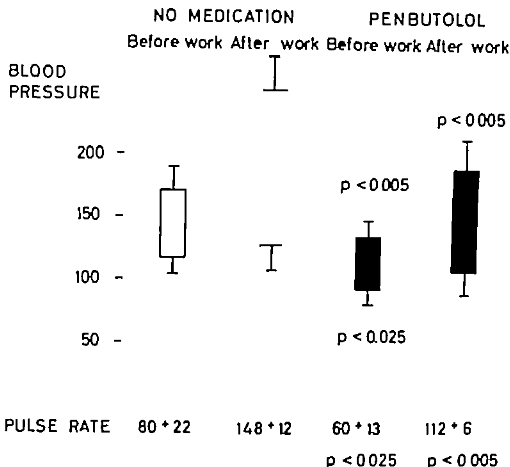


Fig 21 Blood pressure and pulse rate before and during maximal work before and during penbutolol Means ± SD are given

given was determined only in the metoprolol studies and it was found that the reduction in blood pressure was not related to the plasma levels of metoprolol whereas this was the case with respect to the effect on pulse rate Furthermore the blood pressure lowering effect of metoprolol was not related to basal PRA or basal noradrenaline concentrations Also patients with comparatively low PRA showed a good response to penbutolol and metoprolol respectively Thus our findings do not support the view that a decrease in PRA is a primary mechanism whereby beta receptor blocking agents exert their anti hypertensive effect which has been advocated by certain investiga tors (27 28 169) but denied by others (21 77 78) The fact that both penbutolol and metoprolol changed although in opposite directions - the adrenaline response to hypoglycemia which is mediated via central nervous mechanisms seems to indicate that these two compounds exert effects not only peripherally but also centrally It therefore seems

GENERAL SUMMARY AND CONCLUSIONS

The studies presented deal with various effects of long term treat ment with a non selective (penbutolol) and a cardioselective (meto prolol) beta receptor blocking agent in patients with moderate hy pertension Five patients were given penbutolol nine metoprolol For penbutolol the final maintenance dosage was 20-30 mg twice a day for metoprolol 50-150 mg three times a day In the studies with metoprolol investigations were also performed during treatment with placebo The studies included registration of the effect of the re- spective beta receptor blocking agents on blood pressure and pulse rate in supine and upright position and in connection with exercise The influence of medication on catecholamines and renin activity in plasma was studied under the conditions mentioned and also in con nection with insulin induced hypoglycemia In addition the effect of beta receptor blockade on maximal working capacity and basal urinary aldosterone excretion was investigated

Penbutolol and metoprolol both were effective in decreasing blood pressure and pulse rate in supine and upright position and also in connection with submaximal and maximal work The blood pressure lowering effect was not correlated to basal renin activity in any of the two groups nor was it related to basal catecholamine con centrations in plasma

Before medication with beta receptor blocking agents the hyperten- sive patients studied showed the same levels of plasma catechol amines as have been reported for healthy controls Similarly the urinary excretion of catecholamines in these patients were of the same magnitude as in normals

Under basal conditions neither penbutolol nor metoprolol induced any changes in plasma catecholamines in supine or upright position In the penbutolol studies submaximal work caused a minor increase in plasma noradrenaline before medication whereas a definite in

that asthmatic symptoms can occur during treatment with non-selective beta-receptor blocking agents They are considered to result from blockade of the $beta_2$-receptors in the bronchi leading to constric tion (106 107 158)

During penbutolol medication two patients on direct questioning admitted cold hands and feet, although they had not complained spontaneously This side effect probably reflects an increased alfa adrenergic sti mulus (179) During treatment with penbutolol and metoprolol no toxic effects were observed on liver or kidney function nor on blood cor puscles No skin reactions were noted The patients on metoprolol were subjected to specialized ophthalmological examination but no abnormali ties were found

Orthostatism impotence sleep disturbances and/or disturbances in defecation were not observed, neither during penbutolol nor metoprolol treatment

Without medication urinary aldosterone excretion was normal in eight patients somewhat high in five and low in one patient During pen butolol aldosterone excretion was significantly reduced A similar tendency but statistically not significant was seen also during treat ment with metoprolol

During treatment both with penbutolol and metoprolol maximal working capacity was unchanged as compared to before medication but both kinds of medication reduced the increase in blood pressure and pulse rate caused by maximal work

In conclusion the present studies have demonstrated

that moderate hypertension was not primarily related to the production of catecholamines renin and/or aldosterone

that no correlation occurred under basal conditions between the absolute amounts of plasma noradrenaline and the absolute values for plasma renin activity but that a correlation existed between the in crease in plasma noradrenaline and the increase in plasma renin activity on standing

that variations in the concentration of adrenaline in plasma were followed by non-consistent changes in plasma renin activity

that both non-selective and cardioselective beta receptor blockade was followed by a reduction in plasma renin activity in supine as well as in upright position but that this decrease in plasma renin activity was not correlated to the blood pressure lowering effect of the medication

that the decrease in plasma renin activity was correlated to the reduction in urinary aldosterone excretion during beta receptor blocka de indicating a functional relationship between the production of renin and aldosterone eventhough the renin-angiotensin mechanism seemed not to play a dominant role

that both non selective and cardioselective beta receptor blockade caused no changes in the basal catecholamine content in plasma but led to an exaggerated noradrenaline response in connection with sub maximal work which further augmented the alfa receptor predominance produced by beta receptor blockade per se

crease was seen following submaximal work before medication in the metoprolol group Plasma adrenaline did not change following sub maximal work before medication in any of the two groups During pen butolol the noradrenaline response to submaximal work was increased and a definite response occurred also in plasma adrenaline During metoprolol the noradrenaline response was likewise accentuated by submaximal work but there was no increase with respect to adrenaline

Before treatment insulin-induced hypoglycemia was followed by a mark ed increase in plasma adrenaline and a minor elevation of noradrena line in both groups of patients Treatment with penbutolol reduced the adrenaline response to hypoglycemia whereas metoprolol had the opposite effect The noradrenaline response to hypoglycemia was not affected by either penbutolol or metoprolol

Before treatment the basal plasma renin activity was low in two pa- tients within normal range in seven and comparatively high in five In both groups plasma renin levels were significantly higher in up right as compared to supine position prior to medication and the in crease in plasma renin activity on standing was correlated to the con comitant increase in plasma noradrenaline Before medication sub- maximal work caused an elevation of plasma renin activity but this was not related to the increase in catecholamines Penbutolol di- minished the response in plasma renin activity following exercise whereas the effect of metoprolol on the renin response to exercise varied

Before medication insulin induced hypoglycemia was followed by a re sponse in plasma renin activity which varied greatly from one indivi dual to the other Generally the response in plasma renin activity to hypoglycemia was most pronounced in patients with normal or high basal plasma renin activity eventhough the response varied con siderably also in these patients In patients presenting a definite response in plasma renin activity to hypoglycemia before medication treatment with penbutolol caused a diminished renin response The effect of metoprolol on the hypoglycemia induced renin response again varied considerably

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- that treatment with non-selective and cardioselective beta receptor blocking agents was followed by a rather minor decrease in urinary aldosterone excretion and that the blood pressure lowering effects of beta receptor blocking agents hardly can be explained on basis of reduced aldosterone production
- that penbutolol and metoprolol both probably act not only peripherally but also via receptors located within the central nervous system

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New methods for early diagnosis in families with Sipple's syndrome

By Margareta Telenius-Berg

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Ulf Björn Håkan Cecilia
Charlotte and Andreas.

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Acta med scand 196: 177 1974
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III *Serum calcitonin response to induced hypercalcemia A diagnostic aid in early occult medullary thyroid carcinoma*
Acta med scand 197: 367 1975
(In ollaboration with S Almqvist and B Wästhed)

IV reening for medullary carcinoma of the thyroid in families with ipple s syndrome Evaluation of new stimulation tests
Europ J clin Invest 1976 (accepted for publication)
(In collaboration with S Almqvist B Berg P Hedner
S Ingemansson S Tibblin and B Wästhed)

These papers will be referred to by their respective Roman numerals listed above

All k owledg is disturbi g i cludi g tho bo t our g es Do any of u know abo t all ur genetic differenc s and would we be sadder or happi if e did? The question is wheth r on c ld liv with specific knowledg and s it productiv ly

Beck et al (ref 92)

CONTENTS

INTRODUCTION

Our knowledge of medullary carcinoma of the thyroid (MCT) as a distinct clinical and pathological entity is relatively new It was first recognised by Horn in 1951 (1) and further defined in 1959 by Hazard et al (2) in a now classical description of the tumour

In 1961 Sipple (3) showed that the coincidence of thyroid carcinoma and pheochromocytoma was not based on chance concurrence seeing that the incidence was 14 times higher than expected in the general population During the next few years it was recognised that 1/ the thyroid carcinoma in these cases was regularly of the med llary type 2/ a combination with parathyroid hyperplasia or adenoma was rather common and 3/ several members of the same family co ld be aff cted by this tumo r syndrom (4 5 6 7) This regular family pattern indicated that the mode of inheritance was autosomal dominant (8) Oth r tumours of neuroectod rmal origin were sometimes seen in these patients: mucosal n uromas and cutaneous neurofibromas together with a peculiar Marfan-like habitus (9) This tumour syndrome has several denominations: Sipple s syndrome familial chrom ffinomatosis (10) and multiple endocrine adenomatosis (MEA) type II (11)

In 1966 Williams (12) suggested that MCT was histogenetically derived from the parafollic lar cells of the thyroid a fact which is now universally accepted These cells were renamed "C" c lls to associate them with the secretion of calcitonin (13) Evidence that thes cells cont ined calcitonin was provided by immunofluorescence studies in 1967 (14) Subs quently it was shown in 1968 that MCT contained large amounts of calcitoni and that serum calcitoni (S-calcitonin) was raised in patie ts ith thi tumo r (15 16 17 18) The first radioimmunoassay for S-calcitonin analysis in 1969 (19) ope ed th way for a practical clinical diagnosis of MCT of speci l int r st in families with Sipple s yndrome

Th famili s with Sippl s syndrome repr sent high risk populations for th development of MCT and pheochromocytomas At least some family embers die from their hereditary diseas There might therefore be a way to a better prognosis f r th se i di iduals if diagnostic methods

CONTENTS

PATIENT MATERIAL

The Sippl families

My int rest in diagnosing MCT and Sipple s syndrome had its origin in a dramatic clinical experience with the proband of the first Sipple family

Case history (Proband in family I see pedigree I):
In 1968 a 32-year-old woman was admitted to Medical Clinic A University Hospital Lund for investigation of hypertension. She had no known heredity for Sipple s syndrome Her complaints were attacks of retrosternal oppression pounding headache and dizziness. Physical examination revealed a small nodul in the right thyroid lobe and blood pressure 185/120 mm Hg A fine needle aspiration biopsy of the thyroid showed a hitherto unknown cell type dU-VMA (4-hydroxy-3-methoxymandelic acid) was raised 11 mg (normal level less than 7 mg)
A s lective suprarenal angiography was attempted but had to be interrupted because of a hypertensive crisis The following profound hypotension and shock did not respond to the treatment i stituted and the patient died.
Autopsy showed bilateral pheochromocytomas bilateral MCT and a parathyroid adenoma The unknown thyroid cells d tected by the fin needl aspiration biopsy could th s be identified s MCT tumour c lls Thus cytology became a ew diagnostic method which could be used for screening this family for MCT

In 1969 the search for th relatives of this patient began H r mother had been operated on for MCT in 1963 Now she was also shown to have bilateral pheochromocytomas In h r generation littl was known about th broth rs and sisters They had grown up in separat fost r-homes after their mother had died about 30 years old from an unknown ca se Howev r this woman too i e the grandmother of the proband was known to have had goiter The result of th family investigation is shown in the pedigree for family I and in table I

I 1971 the proband of family II ith Sipple s syndrome was found This was on un xpected autopsy diagnosis in a man with remia due to chronic

could be worked out in order to identify these genome carriers and institute treatment as early as possible

AIMS OF THE INVESTIGATION

Development and evaluation of
1/ New methods for early diagnosis of MCT, and
2/ A diagnostic program to be used in screening for hereditary MCT

For this purpose I have studied 59 members of 4 Sipple families, 12 patients with the sporadic type of MCT, and as controls healthy subjects and patients without MCT

genome as based on genetic evidence (Her:3 in pedigree II) and presence of pheochromocytomas (Her:3 7)

2/ Nine family members had <u>previously</u> been operated upon for MCT Some of them had been operated on several times and had received post-operative radiotherapy They had all been followed regularly and in none was there clinical evidence of recurring disease Eight of these nine patients (presented in table III) were found to have raised levels of S-calcitonin This was interpreted as evidence for met stases of MCT

B/ Non-hereditary or sporadic MCT

(For simplicity this patient group is called sporadic MCT although a better denomination would be provisionally regarded as non-hereditary" Only a caref l family screening using sensitive diagnostic *methods will tell for sure if a cas of MCT is sporadic or not. Up* to now I have screened only 3 complete families of these patients) Twelv patients were examined and all of them had their MCT verified by surgery and histopathology Eight of them were studied before treatment and 4 after one or several surgical interventions. (Sex ratio and age distribution is presented in section clinical observations for comparison with the hereditery cases Further clinical data of these patients are considered beyond the scope of the present summary and will be published elsewhere)

Thus the total number of MCT patients studied was 38: the index case of family I 17 ew cases of hereditary MCT as well as 8 patients non-radically operated for familial MCT and 12 patients with sporadic MCT

Borderlin pati nts

Some Sipple relatives (5 individuals in paper III and 3 in paper IV) were cla sified as borderline patients due to the results of the calcium i fusion t st (For a definition of the borderline concept s e paper III)

Healthy Sipple rel tives and control subj ct

According to the results of th screeni g examinations 30 Sipple relatives were provisionally regarded as healthy i e free from detectable MCT

pyelonephritis and nephrosclerosis No one in this family had previously been known to have MCT or pheochromocytoma The screening results are shown in the pedigree II and table I

Family III is a branch of the family reported in 1967 by Ljungberg et al (10) (table I)

Members of family IV were initially examined and diagnosed in 1973 at the departments of endocrinology and surgery Sahlgrenska hospital, Gothenburg Since 1974 further screening of side branches of this family has been performed by the author in collaboration with Dr L Ysander, at the department of internal medicine, Varberg hospital The results achieved up to now are presented in table I
As far as is known these four Swedish families are not related

The individuals and their classification

All the familial cases were members of families I - IV Fifty-nine individuals were examined, viz the index case of family I and 58 first degree relatives of patients with hereditary MCT They were all offered examination as part of the screening program The results are presented below, in table I - IV and in pedigrees I - II in this summary For clinical data see also section "Clinical observations" To avoid the possibility of personal identification individual data concerning the operative findings and the results of treatment are omitted in table II

MCT patients

A/ Hereditary MCT

1/ Seventeen new cases of MCT were diagnosed as a result of the screening procedure None had sought medical attention because of symptoms referring to the thyroid gland Fourteen of these patients have subsequently been operated upon for MCT (See table II) In 3 patients the diagnosis has not yet been verified by histopathology One patient is awaiting surgery (patient Her:13) one patient refuses surgery as he feels well (Her:3) and the third patient (Her:7) is being followed elsewhere The MCT diagnosis in these three cases is based on raised levels of S-calcitonin Two are also proved carriers of the Sipple

SCREENING PROGRAM

Fine needle aspiration biopsy was our first method for preoperative diagnosis of MCT It was applied together with the earlier well known conventional methods 1 and 2 (see below) which however are not specific at all for MCT Thus my first screening program included:

1/ Clinical examination including careful palpation of the thyroid by at least two experienced physicians
2/ Thyroid scintigraphy using either ^{131}I or ^{99m}Tc-pertechnetate
3/ Fine eedle aspiration biopsy
4/ Chemic l analyses: B-hemoglobin B-leukocytes S-creatinine S-calcium S-phosphate dU-VMA (20) dU-methoxycathecholamines (21) dU-adrenaline and dU-noradrenaline

When this screening program was applied to Sipple family I 2 new cases of MCT and 2 new cases of pheochromocytoma were diagnosed One case of sporadic MCT was also detected by cytological examination In 1970 we reported our preliminary results of this diagnostic method for MCT (22) During the following years the rapidly i creasing patient material yielded extended experience The results are presented in paper I

In 1970 our radioimmunoassay technique for S-calcitonin d termination was introduced in clinical practice (paper II) It was soon realis d that this method was more sensitive than cytology for detecting very small non-palpabl tumours (See section Clinical observations) Ther fore the screening program was somewhat modified: In patients without palpable abnormalities and with n rmal S-calcitonin thyroid scintigraphy and aspiration biopsy were omitted If S-calcitonin later increased as in some of the MCT patients a thyroid scintigram was performed. (The results of the isotope studies includi g also ^{67}Ga and ^{137}Cs will be presented seperately)

Of course, these relatives may still carry the Sipple genome although as yet without having any detectable tumours Another designation for this group is therefore "high risk individuals which was chosen in paper IV to avoid the ambiguous word "healthy"
In addition a number of healthy subjects and patients with various diseases (paper I: goiter not caused by MCT, paper III: hypocalcemic paper IV: symptoms of gastric ulcer) were studied as controls (See papers I-IV)

The patients have all been examined during the 6-year-period 1970-1975 with varying observation times During these years the diagnostic methods have been developed Therefore not all patients have been examined with all the new methods and only some patients appear in all the papers I - IV A few patients are presented preoperatively in studies I - III and postoperatively in the last study IV

The evaluation of each separate method is based on the patient material presented in the corresponding paper However, the longitudinal study has provided material for comparing the methods with each other

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All adult patients were examined clinically and the basal level of S-calcitonin determined Most adult patients were also submitted to one or several of the stimulation tests with calcium, pentagastrin, cholecystokinin-pancreozymin and ethanol A modified program was used in children No child was less than 7 years at the first examination Children 7 - 14 years old were not examined with stimulation tests or thyroid scintigraphy This cautious standpoint was adopted pending further experience (In 1975 only 4 of the children in this patient material still belonged to this age group)

METHODS AND RESULTS

Fine needle aspiration biopsy with cytological examination (Paper I)

Method

All palpable and/or suspected thyroid and lymph node abnormalities were biopsied The aspiration biopsy was performed according to Söderström (23) with one-hand-syringes and disposable needles with an outer diameter of 0 6 mm *The aspirates were smeared on glass slides* These were either air-dried for staining with May-Grünwald-Giemsa (MGG) or fixed in 80% ethanol for staining with hematoxylin-eosin or alkaline Congo red

Results

A/ Characteristic morphological features of MCT tumour cells after MGG staining:

The tumour cells varied considerably in size but were usually larger than the normal follicular cells Multinuclear giant cells were not uncommon Quite often the shape was characteristically triangular or spindle-shaped with cytoplasmatic "dendrite-like" extensions The cytoplasm was amphophilic and structureless in most cells However in a certain proportion usually 5 - 10% of the total tumour cell population a distinct red granulation was seen The granules were often rather coarse but sometimes fine and dustlike These granules did not take stain with eosin alone Thus this eye-catching detail of MCT cytology is lost in smears stained with hematoxylin-eosin

The nuclei were nearly always excentrically positioned in the cells dark and sharply outlined Only rarely were distinct nucleoli seen A high proportion of the cells were characteristically binucleate Amyloid was seen as amorphous lumps usually extracellular but sometimes also as small

intrecytoplasmatic inclusions The amyloid stained greyish blue or violet with MOO and bright red with hematoxylin-eosin With alkaline Congo it was light red in common light microscopy but showed the characteristic green birefringenc in polarised light
Biopsy material sufficient for staining with alkaline Congo was obtained in 14 MCT patients. Nine of these were positive for amyloid To study the specificity of amyloid in MCT the author examined 60 fine needle aspirates from 30 consecutive patients with miscellaneous types of goiter other than MCT In none of these controls could any extra- or intracellular lumps of amorphous substance with amyloid criteria be found.

B/ Diagnostic results
Eighteen patients with MCT were examined. In the 13 MCT patients with a palpable neck abnormality the biopsy was positive for MCT in 12, i e a correct diagnosis was obtained in all cases but one A false negative result was obtained in a woman with a small soft nodular goiter (She is one of the three patients who have not as yet been surgically verified) Probably the palpable abnormality in this patient was not caused by MCT which should be small according to the only slightly increased level of basal S-calcitonin 2.2 ng/ml. (Upper normal level (mean + 2 S.D) 1 0 ng/ml Paper II)
In the other 5 patients punctures were performed against the thyroid region without a convincing palpatory target In only two of these was th bioptic yield sufficient for evaluation giving normal cytology in one and MCT cells and amyloid in th other Th s in 5 patients <u>without</u> a palpable thyroid or lymph node abnormality the correct diagnosis was obtained in only one case The conclusion may be drawn that <u>in most</u> patients with an MCT l rge enough to be palpable the correct diagnosis sho ld be obtained by cytology

S rum c lcitonin in dull ry carcinoma Radioimmunoassay technique and diagnostic value (Paper II)

<u>M thod</u>
A radioimmunoassay for an lysis of S-calcitonin in untreated human serum was dev loped Th best anti erum was obtained from a rabbit immunised ith xtr ct from a human amyloid-producing MCT together with Freund s adjuvance The antis rum as sed in final dilution of 1:900.

All adult patients were examined clinically and the basal level of S-calcitonin determined Most adult patients were also submitted to one or several of the stimulation tests with calcium, pentagastrin, cholecystokinin-pancreozymin and ethanol A modified program was used in children No child was less than 7 years at the first examination Children 7 - 14 years old were not examined with stimulation tests or thyroid scintigraphy This cautious standpoint was adopted pending further experience (In 1975 only 4 of the children in this patient material still belonged to this age group)

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Serum calcitonin response to induced hypercalcemi (Paper III)

I patients with very small MCT tumours the determination of basal S-calcitonin may not be sensitive enough to give diagnostic results (27) If the C cells could be provoked to a sudden release of calcitonin this might provide us with an even more sensitive method that would disclose occult i e small asymptomatic tumours (28) We first chose induced hypercalcemia as a stimulus as it is well established that the secretion of c lcitonin is regulated directly by the blood calcium concentration (29 30,31)

M thod

We measured the change in S-calcitonin induced by transient hypercalcemia duri g i.v infusion of calcium gluconate (0 375 mmol 0 75 mEq = 15 mg/kg body weight/4h) in 10% invertose solution totally 1000 ml All healthy controls and all patients but one were fasting S-calcitonin was analysed in peripheral venous blood taken at 0 60 and 240 min In some patients additional samples were collected at 30 90 120 and 180 min Zero time (0 min) was the start of the infusion usually at about 10 a m S-calcitonin at 0 min (i e the basal or non-stimulated S-calcitonin) was the rithmetic mean of two separat samples taken at -5 min and just before the start of the infusion The change i S-calcitonin during the induced hypercalcemi at t min aft r z ro time was denoted ΔCT_t (In paper IV ΔCT_{240} was designated ΔCT_{Ca})

Res lts

14 MCT patients 19 clinically healthy Sipple relatives 16 healthy controls and 2 patients with chronic hypocalcemia (due to intestinal diseases) wer studied.

Most MCT patients chieved their maximum S-calcitonin level at the end of the infusion i $\Delta CT_{240} > \Delta CT_{60}$ (table I and fig 3 in paper III) For f rther studies we chose ΔCT_{240} as our diagnostic parameter In 13 MCT patients (in one patient th serum sample taken at 240 min was lost) ΔCT_{240} varied from 2.2 - 630 ng/ml i.e markedly exceeding the ref ren range for healthy controls: -0 2 — +0.5 ng/ml (mean ± 2 S.D) (fig 1 in paper III)

In both patients with hypocalcemia ΔCT_{240} was normal

Fourteen out of 19 clinically healthy Sipple relatives (i e without signs of thyroid tumour or pheochromocytoma) had normal basal S-calcitonin

Synthetic human calcitonin M (CIBA) was used for labelling with ^{125}I (Amersham England) with a modified chloramine-T method according to Greenwood and Hunter (24) The synthetic human calcitonin M was also use as a reference standard The quality of the labelled antigen was tested with chromatoelectrophoresis (25) Calcitonin-free serum was obtained from a woman who had been completely thyroid- and parathyroidectomised for a mixed follicular and papillar thyroid carcinoma Separation of bound and free hormone was accomplished by the dextran-coated charcoal method (26) Unknown samples were always run in duplicate (and standards in triplicate) and often in multiple dilutions to check that the plotted results would fall along the standard curve

Results

In the assay the antiserum was specific for human calcitonin The dilution curve for the measured activity in serum from MCT patients parallelled the corresponding curve for synthetic calcitonin M (fig 1 in paper II) Thus we could not find any detectable difference in immunochemical behaviour between endogenous hormone in serum and the synthetic calcitonin standard No cross reaction occurred even with large amounts of human and bovine parathyroid hormone, human adrenocorticotropin and human somatotropin

The coefficient of within-batch variation was 14% at a S-calcitonin level of 10 ng/ml and for between-batch variation 12% at 3 ng/ml

Reference values for S-calcitonin in healthy controls (94 adults and 21 children) were 0 70 ± 0 34 ng/ml = 0 20 ± 0 10 nmol/l (mean ± 2 S D) There was no relationship to age or sex The detection limit of the method was 0 40 ± 0 20 ng/ml = 0 11 ± 0 005 nmol/l For conversion into SI units, 1 ng/ml calcitonin = 1 μg/l = 0 28 nmol/l, the molecular weight for synthetic human calcitonin M, 3 HCl being 3,527

MCT patients: S-calcitonin levels were raised in all of the 21 examined patients with verified MCT, ranging from 1 6 up to more than 800 ng/ml (fig 2 in paper II) There was a good correlation between the S-calcitonin level and the clinically estimated tumour cell mass Of the 21 MCT patients 16 had a palpable neck (thyroid and/or lymph node) abnormality, whereas 5 had no abnormal palpatory findings at all No false positive results were seen in this material, i e in all patients with a raised level of S-calcitonin the presence of MCT could be verified by histopathology

4/ The report of Cohen et al that whisky induced a rise in S-calcitonin in one patient with MCT (36)

Method

The rise in S-calcitonin induced by pentagastrin cholecystokinin-pancreozymin and ethanol was measured

1/ Pentagastrin (PG) (Peptavlon ICI England) was given as a s.c. (6 µg/kg body weight) or a rapid i.v (0.6 µg/kg body weight) injection.

2/ Cholecystokinin-pancreozymin was administered in two forms:

a/ the synthetic C-t rmi al octapeptide CB-CCK (a gift from Dr Ondetti Th Squibb Institute for Medical Research USA) 6 6 µg was given as a slow i.v injection during 5 min

b/ the purified native porcin extract CCK (a gift from Drs Jorpes and Mutt Gastrointestinal Hormone R search Unit Karolinska Institute Sweden was injected i v d ring 2 min. 1 Ivy dog unit/kg body weight

3/ Ethanol stimulation was performed according to Cohen et al (36) 45 ml whisky (43% ethanol v/v) was given orally to the fasting subject (one of th se patients was given 90 ml on a separate occasion)

S-calcitoni was analysed in peripheral venous blood taken 15 min before (-15) just b fore (0) and 2 5 10 15 30 45 60 90 and 120 min fter the administration of the stimulating agent The mean value of the two determinations at -15 and 0 mi was taken s the basal i e non-stim lated S-calcitonin

R s lt

The ma imum ris in S-calcitonin induced by thes agents was designated ΔCT_{PG} ΔCT_{CB-CCK} ΔCT_{CCK} a d ΔCT_{Et} with the indices (s c.) and (i) to i dicate the way of administration of pentagastrin (I paper IV ΔCT_{Ca} is equivalent to ΔCT_{240} i paper III)

After c pentaga tri most pati ts chiev d th ma imum level of S-calcitoni at 5 i nd after i pentagastrin at 2 mi For the other agents th ae k vol es wer always reached within 15 min and mostly thin 5 i (fig 2 i pap r IV) As compared to calcium all th se agents induced a ry r pid and brief r leas of calcitonin (I a calcium i f sion t at th ma i um S-calcitoni level was us ally eached at th

and ΔCT_{240} levels within the normal range In the other 5 Sipple relatives ΔCT_{240} fell within a range between that of healthy controls and that of verified MCT (fig 1 in paper III) Two of these also had slightly elevated basal S-calcitonin levels (table IV in paper III) According to the results of the calcium infusion tests these 5 patients were called "borderline patients"
About one year later they were reexamined (table IV in paper III) At this second examination the ΔCT_{240} levels were found to have increased in 3 out of 5 patients, thereby passing into the range for verified MCT When compared to basal S-calcitonin ΔCT_{240} was found to have a better discriminating ability The deviations from normal were greater ex pressed both in absolute concentrations of S-calcitonin and in standard deviations from the normal mean (fig 5 in paper III)
Since the publication of paper III the other two borderline patients have also developed levels of ΔCT_{240} consistent with MCT Four of the 5 border line patients have subsequently been operated and the MCT diagnosis verified by histopathology The fifth patient is awaiting surgery
Side effects of the calcium infusion: Slight nausea in a few subjects

Evaluation of stimulation tests with pentagastrin cholecystokinin-pancreozymin and ethanol (Paper IV)

The calcium infusion test had been shown to be a highly sensitive diagnostic method for MCT However to allow a widened use in clinical practice a simplification of the stimulation test would be desirable Therefore I tested gastrointestinal hormones and ethanol as stimulating agents There were several reasons for this choice:

1/ Calcitonin release had been found in pigs after administration of pentagastrin (a synthetic modified C-terminal tetrapeptide of gastrin) and cholecystokinin-pancreozymin (both the native hormone and the synthetic C-terminal octapeptide, C8-CCK) (32 33) Actually all these three substances have the same C-terminal amino acids

2/ Our own studies in 1973 of gastric secretion in MCT patients after administration of pentagastrin (The secretion of hydrochloric acid is inhibited by calcitonin (34))

3/ The report in 1973 of Hennessy et al that i v pentagastrin induced a pronounced and rapid increase of S-calcitonin in two patients with MCT (35)

Side effects of C8-CCK and CCK: During the injection all subjects experienced abdominal cramps which were somewhat more pronounced than after pentagastrin

3/ Ethanol

ΔCT_{Et} was clearly raised in 3 of 5 MCT patients whereas it was just above the upper normal limit in the other two In one of the MCT patients a double dose of whisky gave a S-calcitonin response that was three times higher (table IV in paper IV)

Comparisons between different stimulation t sts

A good correlation was obtained between the results after stimulation with s.c. pentagastrin and hypercalcemia but the effect of C8-CCK and ethanol was generally weaker (See fig. 3-5 in paper IV) Furthermore C8-CCK gave a variability in the response that was not seen in corresponding trials with pentagastrin

Conclusions:

Pentagastrin was th best of the new agents tested and the results comparable with our best method so far the calcium infusion test The s c administration of pentagastrin is used in many hospitals for stimulation of gastric secretion of hydrochloric cid For studies in Sipple families the i.v pentagastrin stimulation t st should principally be preferred as it gives a more i tense release of calcitonin

The cl ssification of the subjects into diagnostic groups was not changed if only two blood samples at 0 and 5 min were used for calculation of ΔCT_{PG}(s c.) (0 and 2 min for ΔCT_{PG}(i v)) Thus only th se two blood sample a nece sary for diagn sing MCT in asymptomatic patients with sm ll tumours and normal levels of basal S-calcitonin

end of the 4 hour infusion, i e at 240 min (fig 3 in paper III) The effects of the different stimulation agents were compared with one another and with our best diagnostic method so far, i e the calcium infusion test

Results of individual stimulation tests

1/ Pentagastrin Thirtytwo stimulation tests were performed with s c pentagastrin in 18 MCT patients Only one of these had a palpable tumour (table I in paper IV) Seventeen of 18 responded to pentagastrin with a rapid increase in S-calcitonin which far exceeded the highest value in the controls (fig 1 in paper IV) The total range of ΔCT_{PG} (s c) among these 17 responders was 1 0 - 630 ng/ml The reference range for the controls was 0 09 $\pm$ 0 22 ng/ml (mean $\pm$ 2 S D) (See table II in paper IV) There was generally a good correlation between ΔCT_{PG} (s c.) and tumour cell mass as estimated at operation

I v pentagastrin was tested only in 4 patients and induced a more intense stimulation of calcitonin release than did s c pentagastrin (table II in paper IV) The MCT patient who had a false negative" result after stimulation with s c pentagastrin had a raised ΔCT_{PG} (i v), which was about equal to ΔCT_{Ca} in the same patient

Three "borderline patients (according to the criteria in paper III) were examined with the s c pentagastrin test In one of these ΔCT_{PG}(s c) was clearly elevated into the range of the pentagastrin-responsive MCT patients (MCT has later been verified in this patient) Thus, in this case pentagastrin was more efficient than the calcium infusion test in establishing the correct diagnosis On the other hand in the other two borderliners there was no response to pentagastrin (table II in paper IV) Side effects of pentagastrin: All subjects experienced abdominal and/or precordial distress lasting less than one minute after i v and less than two minutes after s c injection One young woman fainted in the lying position

2/ Cholecystokinin-pancreozymin (C8-CCK and CCK)

Twelve tests with C8-CCK were performed in 9 MCT patients two of whom were also examined with the native porcine extract (CCK) All MCT patients had increments in S-calcitonin after C8-CCK exceeding the highest value observed in the control group and so had two borderline patients CCK induced a similar response as did C8-CCK (table III in paper IV)

comparing the different diagnostic methods with each other The results of scintigraphy are therefore presented in table II

Scintigraphies were performed in 16 of the 17 new patients with MCT In one patient the thyroid uptake was too low to permit diagnostic conclusions The res lts of the scintigrams in the other 15 patients were called either bnormal uncertain or normal The scintigram was judged as abnormal if there were on or several well demarcated areas ith red ced uptake of the isotope as compared to the other parts of the thyroid. The scintigram was called uncertain if the radio-uclide uptake was irregular generally or locally Finally the scintigram was called normal if the uptake of isotope was even in thyroid lobes of normal siz

Eight of 15 scintigrams were judged as abnormal 4 wer judged as uncertai and 3 were normal

However the interpretation of scintigraphy is often difficult In the absenc of palpable abnormalities areas of slightly irregular uptake may be diffic lt or impossible to evaluate The scintigrams were judged by different physicians. In addition the interpretation of som scintigrams in the present series was bi sed by the fact that the results of other investigations were lready known

I cidence of pheochromocytoma (Tabl I pedigree I and II):

Seven ew cases were found through the screening program and 5 of th se have been verified by surgery and histopathology In the two non-operated patients the diagnosis was based on raised levels of dU-cathecholamines and -metabolites. On c se also had a positive result of selective suprarenal angiography This patient does not want surgical treatment as he is completely symptomatic and the other one is considered inoperable due to coronary heart disease

Characteristic paroxysmal symptoms of thes adrenal tumours had been present for several years in three of the operated patients found during the family screening In none had the right diagnosis been suspected. One 52 year-old woman was traced only after sh had a myocardial infarction She had then hypertension with a resting blood pressure of 250/120 mm Hg between the hypertensive attacks.

CLINICAL OBSERVATIONS

Sex ratio (Table II):

The sex ratio among the familial cases of MCT was 1 2:1 (males/females 14/12) and among the sporadic MCT patients 0 7:1 (males/females 5/7)

Age at diagnosis (Table II III and fig 1):

Seventeen new cases of familial MCT were detected by the screening program Their age at diagnosis ranged from 15 to 58 years the majority (11 patients) being diagnosed between 21 and 30 years (Fig 1 A)

Nine patients had previously been operated for familial MCT The tumour had then been detected only after the manifestation of clinical symptoms Patient age at diagnosis ranged from 36 to 51 years (Table III) Thus it is apparent that the screening procedure leads to earlier detection of the tumour in families with Sipple s syndrome (Fig 1 B)

As in the second group above sporadic MCT is usually diagnosed only after the presence of a neck tumour has brought the patient to a doctor The patient age is then still higher than in the other two patient groups (fig 1 C)

Results of physical examination (Table II):

Only 6 of the 17 new cases of familial MCT had a palpable thyroid abnormality In 11 cases palpatory findings were considered normal by at least two experienced physicians None of the 17 had palpable lymph node metastases

As compared with determination of basal S-calcitonin physical examination had a lower specificity as well as a lower sensitivity as regards the MCT diagnosis All 21 MCT patients in paper II had raised levels of S-calcitonin whereas only 16 had palpable neck abnormalities Thus, 5 cases were misdiagnosed on physical examination

Results of thyroid scintigraphy (Table II):

The special problems of thyroid scintigraphy are beyond the scope of this work Therefore the results of this part of the investigation will be presented as part of a separate study (37) However the practical results of the scintigraphy performed in these patients are of interest when

The end results of treatment are difficult to foretell and two patients exemplify this: One patient diagnosed and operated for MCT at the age of about 20 had normal results of calcium infusion tests during the first 4 years after operation. Then the S-calcitonin response to calcium and pentagastrin increased beyond the normal range which was interpreted as indicating slow growth of residual MCT
Another young patient of about the same age had no clinical signs of tumour on clinical examination before operation However the S-calcitonin levels were very high Therefore it was not unexpected to find extensive growth of metastases in neck and mediastinal lymph nodes According to the results f regional analysis of S-calcitonin after selective venous cath terisation (performed postoperatively) this patient has also hepatic metastases.

The results of treatment so far may be compared with the results in their relatives who had been operated earlier for MCT In the latter group only 1 of 9 patients was free from tumour according to the res lts of S-calcitonin determination However the groups are not quit comparable as the mean observation time is much longer in this latter group

The family histories show that several rel tives of the patients in this terial hav indeed died from their MCT usually in their fifth or sixth d cade The youngest was a man who died 42 years old Other have died suddenly from their pheochromocytoma as did the inde case in family I and 42-year-old woman in family II
In this patient mat rial it is impossible to ascertain the true incidence of deaths du to MCT or pheochromocytoma This is due to the fact that th caus of death was not c rtified by utopsy in most relatives who deceased bef r 1960 F rthermor MCT was not known as a cli icopathological entity befo e this time

Other clinical manifestations of the Sipple syndrome

Parathyroid adenoma was diagnosed (at autopsy) in one patient the index case of family I One of the 14 new cases operated for HCT had hyperplasia of one parathyroid gland No patient had clinical symptoms and signs of hyperparathyroidism, neurofibromas or neuro-gangliomas Three patients had the typical Marfan-like physique

Result of surgical treatment in the new cases of hereditary HCT:

The surgical aspects of the treatment of HCT will be the topic of a separate study A preliminary report has been presented in 1975 (38) However, the results of treatment and especially the degree of radicality are of interest in this study as they seem to be related both to patient age and tumour size at the time of diagnosis (Table IV A - C)

The provisional opinion of radicality has been based not only on histopathological examination of the surgical specimen but also on determinations of S-calcitonin during stimulation tests with calcium and pentagastrin These tests have usually been performed regularly first a few days after operation and then at 6- or 12-monthly intervals
The observation time after operation naturally varies 2 patients have been followed for 5 years 3 for 3 years and the remaining 9 for less than 3 years

Table IV:A-C shows the result of treatment as related to clinical findings, i e age at operation, palpable abnormalities on physical examination and tumour size as measured at operation
Three of 11 patients less than 30 years old could not be radically operated (table IV:A) Obviously they were diagnosed too late However, generally the chance for definite cure is greater the smaller the tumour size as estimated clinically and at operation (table IV:B and C) This gives support to the view, that these patients should best be diagnosed and treated as early as possible in the evolution of the tumour Two of 14 patients had local lymph node metastases (found at operation and verified by histopathology)

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DISCUSSION

I Evaluation of the new diagnostic methods

In a recent review Sackett (39) points out certain properties of clinical diagnostic methods for screening purposes: 1/ acceptability (the extent to which the volunteer or the patient is willing to undergo the test) 2/ simplicity (the ease of performance of the test) 3/ cost 4/ precision (the consistency of repeated measurements) 5/ accuracy (the closeness to the true measure of the attribute being sought) 6/ sensitivity (yielding a positive test result in a high proportion of individuals with the disease) 7/ specificity (yielding a negative test result in a high proportion of individuals who are free from the disease and 8/ predictive value (resulting in high proportions of diseased and disease-free individuals among those with positive and negative test results respectively)

The relative importance of these properties naturally varies It is related both to the purpose of the investigation and the calculated clinical benefit of a positive diagnosis in the individuals tested According to my experience the members of Sipple families are generally well motivated for examination once they have been informed about the nature of the disease In order to perform repeated controls, possibly over a period of many years it is important that the acceptability of the test is high Furthermore the examination must be simple and practical both for the patient and the medical personnel involved Also the cost must be judged in relation to the clinical benefit The cost aspect will not be discussed here As to points 4 - 8 the degree of sensitivity specificity and predictive value are judged to be the most important properties for screening purposes To this I would like to add another important quality For use in the screening of subjectively healthy asymptomatic persons the test used must be unatterded by risks for complications
When trying to evaluate the respective methods regarding these properties each method will be discussed separately

A/ Fine needle aspiration biopsy

We found that the cytological features of MCT were generally highly characteristic and easily recognized In thyroid tumours the demonstration of

amyloid is specific for MCT However the total amount of amyloid may be small and the distribution is often uneven and patchy Therefore this component may escape detection in cytological specimens as well as in histological ones

The red granulation though a highly characteristic feature of MCT is not entirely specific. It is noted that similar red granulated cells are seen in other tumours of neuroectod rmal origin: pheochromocytoma chemodectoma and carcinoids The granulation is also akin to that seen in some mammary carcinomas It is of theoretical as well as of practical interest that calcitonin production has been shown notably in pheochromocytomas (40 41) carcinoids (42 43) and quite recently also in mammary carcinomas (44,45) The red granules seen in MGG stain in common light microscopy might thus be con ected with the prod ction of calcitonin or similar polypetides

Although the diagnosis of MCT in one of our patients (patient A:10 in paper I) was obtained as th result of a blind" puncture (h had no palpable thyroid abnormality) we do not generally advocat or perform thyroid biopsies in s ch cases. In these cases the immunological determination of S-calcitonin has replaced cytology as the method of choice for screening primarily because of the much high r sensitivity

But in patients in whom the MCT is large enough to be palpable the odds ar very high (12/13 92%) for a specific di gnosis of MCT (provided the technical quality of the smear is good) I other words: the sensitivity of the method is high in th s c ses

There are sev ral practical advantages of fine needle aspiration biopsy of thyroid abnormalities the technique is relatively simple and quick (the smear can be stained and xami ed while the patient is waiting) F rthermore si ce it caus s n gligible discomfort to the patient it can be r peated several times if the bioptic yield is not adequate Local aneth sia is not req ired Complications r rarely seen (at most a small haemorrage)

Of cour ther ar also som disadvantag s:

1/ It is importa t that the t chnical quality of the smea is high Some traini g i th punct r and smeari g techniques is essential Thus the di gno tic yi ld is increased if the biop y and th cytological e ami ction are performed by one and th same person I Sweden this is no great problem

as there are departments of clinical cytology at many hospitals Elsewhere, however, this technique is still rather uncommon Like others (46) we believe that this is a coming technique in other countries also

2/ The risk of spreading malignant cells during the aspiration biopsy This risk is probably relatively small (47)

Generally the diagnostic advantages of cytology in the clinical evaluation of thyroid nodules by far outweigh the disadvantages The diagnostic alternative — a surgical biopsy — implies the same or possibly even higher risk for haemorrage and spread of malignant cells

B/ Determination of S-calcitonin basal and post-stimulation levels

In 1970 determination of S-calcitonin was introduced as a diagnostic test for MCT It seemed to be a tremendous advance if a blood analysis could tell whether a Sipple relative had MCT or not Relatively soon it became obvious, that the method was superior to our best method so far, i e fine needle aspiration biopsy Thus, by this contribution to our diagnostic arsenal small tumours of only rice grain size could be detected and treated.

However, this method also introduced new diagnostic problems What should be done with patients with basal S-calcitonin near the upper normal level? How much could we trust a chemical analysis with our limited clinical experience? We did not want to perform explorative or diagnostic thyroidectomies in these usually young patients To go further calcium infusion tests were performed in most of these patients Primarily, however, this rather time-consuming test did not seem to contribute much to the diagnosis. In all MCT patients both basal S-calcitonin and ΔCT_{240} were raised (Fig 2 in paper III)

However with this test 5 out of 19 clinically healthy Sipple relatives were distinguished This new test thus seemed to be a diagnostic advance A continuous follow up of these 5 patients gave definite proof for this assumption as all 5 have developed ΔCT_{240} levels consistent with MCT Four of them have subsequently been verified by surgery and histopathology This experience has taught us that 1/ patients with early MCT may have normal levels of basal S-calcitonin and that 2/ our borderline range for suspected MCT represents an early neoplastic stage of the disease

The interest for Sipple s syndrome and the diagnostic use of S-calcitonin determination has evolved independently but almost parallell here in Sweden and in USA. Also our clinical results discussed above are almost identical ith those of Melvin et al (48 49) and Block et al (50,51) These two groups used the radioimmunoassay technique for S-calcitonin of Tashjian (52) which seems to have about the same sensitivity as ours in the clinical situation Other groups had a higher percentage of false negative results probably due to less sensitive radioimmunoassay methods (53,54) For diagnostic purpose it is important to define and standardise the Δ-concept ith statement of the reference values found g. in healthy controls.

The sensiti ity of our calcium infusion t st suffices well enough to diagnose even very small tumours, Thus it is sensitive enough to have a value in the clinical diagnostic situation but not for physiological studies in healthy controls This latter purpose demands a very high sensitivity and precision of the analytical method for S-calcitonin The calculation of Δ-values involves a subtraction of one calcitonin concentration from another This may mean the addition of two small inaccuracies i the methodological error for ΔCT_{240} is double that for the S-calcitonin method.

I soon had a rapidly increasing number of patients in n ed of regular examinations for MCT This necessitated a search for a simpler and less laborious method than the calcium infusion test Th refore we tried other stimulators of calcitonin secretion: pentagastrin cholecystokinin-pancreozymin and ethanol (paper IV)
A good corr lation as regards th sensitivity was found between the results of the calcium infusion test and the s.c pentagastrin test Thus it might be j stifi ble to replace th calcium inf sion test with the much simpler and quicker pentagastrin test.
The i v pentagastri test was evaluated only in a limited number of patients The S-calcitonin response here was somewhat stronger than to s c. pentagastrin indicating that the i v administration should be preferred. On the other hand the reason for primarily choosing s c. pentagastrin was that this administration f rm was already well known and accepted as a routine in most Swedish hospitals. This could possibly facilitate the decentralisation of MCT diagnosis and follow-up So far our practical xperienc has lent support to this assumption

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Furthermore there is the practical disadvantage with alcohol that it is less suitable in car-driving patients Nor have we found any other advantages that might compensate for the lower stimulating effect. However another Swedish group has had satisfactory diagnostic results when using S-calcitonin determination after alcohol for screening purposes in a Sipple family (59)

Considerations regarding sensitivity and specificity:

Different investigators report somewhat conflicting results regarding the S-calcitonin levels in health and in different pathological conditions (52,60 61 62 63) Thus usually normal basal lev ls are reported to be less or much less than 1 ng/ml with varying lower detection limits. However these numerical values although of theoretical interest are of less clinical relevance The important point is the degree of overlapping between individuals without MCT and patients with this neoplasm The cut-off point between normal and suspected disease may vary depending on the purpose of the investigation which will modify the need for sensitivity and specificity For my purpose I have chosen mean + 2 S D

A low sensitivity will lead to a high percentage of false negative results i e MCT patients will show up with normal S-calcitonin levels. Like other authors (52) we found a correlation between serum levels of calcitonin and stimated tumour cell mass This means that an assay with low sensitivity will misdi gnose patients with even relatively large tumours whereas with a high-sensiti assay only sm ller tumours will be missed The stage of the disease and the estimated tumour mass must be properly presented if one is to be able to evaluate the sensiti ity of the m thod If false negative results are found in MCT patients we m st know how the diagnosis was est blished and the size of the tumour

I y first clinical materi l (paper II) all patients operated upon and verified as MCT had rai ed levels of basal S-calcitonin Th s there were no f lse egatives. Som years after th i troduction of stim lation t sts I was able t find si gle cas s with ormal S-calcitoni and MCT which in thes cases was only a few mm in size Cert inly there are false negati e cas s l o after stim lation tests (Th young patient with normal values of ΔCT_{240} for 4 years aft r s rgery and then slightly raised levels

Other reports have confirmed our results as to the pentagastrin test as compared with the calcium infusion test (55,56,57) Thus, up to now, most authors agree that the pentagastrin test is as good a stimulator or even better than the calcium infusion test *In addition it has many practical* advantages: it is quick (we need only two samples of venous blood drawn within a few minutes) and it is simple (involving only a minimum of medical personnel) The patient s subjective discomfort is usually mild and will pass away within a minute (All patients, who had experienced both the calcium infusion test and the pentagastrin test preferred the latter mainly because of the time gained)

One question deserves consideration as to the pentagastrin test As yet we do not know if pentagastrin might stimulate the release of catechol amines from a pheochromocytoma and thus induce a hypertensive crisis or cardiac arrythmias Further studies are needed in order to rule out this possible risk Only 2 patients in this material (patients A:1 and A:6 in *paper IV) had pheochromocytomas at the time of the pentagastrin test* In neither of these did the injection of pentagastrin provoke symptoms that might indicate a massive release of catecholamines On the other hand 6 patients (patients I:1, I:2, II:1, II:2, II:3 and III:3 in paper III) had pheochromocytomas at the time of the calcium infusion test In none of these did the calcium infusion test arouse symptoms or adverse reactions like those known to be caused by the release of catecholamines

To the best of our knowledge there is no other report of *cholecystokinin* as a calcitonin releasing agent in MCT patients We could not find any practical advantages with C8–CCK as compared with pentagastrin

None of our MCT patients reported any adverse reactions (cutaneous flush, tachycardia and abdominal colic) after ingestion of alcohol Such symptoms were noted in the one patient reported by Cohen et al (36) Regarding the clinical value of this test, we found it to be inferior to the pentagastrin test The same results were recently reported by Milhaud et al (58)

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indicating residual tumour, is an example Thus, to evaluate fully the sensitivity of our own diagnostic methods, it was necessary to follow a large patient material for several years If this is not done, the true number of false negatives is difficult to ascertain as long as we do not perform "diagnostic" thyroidectomies on all Sipple relatives Naturally we realize that a "diagnostic" or "explorative" thyroidectomy would give a positive result in almost 50% if all first degree Sipple relatives were operated on Even if actual MCT was not already present, there would be a diminution of the developmental potential of the tumour to come However, an operative exploration without removal of the gland would not be enough In the very early cases the MCT is so small and difficult to find even with bidigital palpation in the wound, that the gland must be sectioned to find the tumours
Up to now we have taken a cautious stand in order not to operate unnecessarily We have therefore required high or rising values of ΔCT_{240} or ΔCT_{PG} This was also necessary in order to verify that the borderline levels of ΔCT_{240} represent a preneoplastic or early neoplastic stage The fact that we have been able to verify the MCT diagnosis by histopathology in all operated cases has contributed to an increased confidence in the method

During the past few years evidence has been accumulating that high levels of S-calcitonin, as determined by radioimmunoassay are not entirely specific for MCT Such increased levels may occur in a number of other conditions These are:

1/ Non-MCT tumours notably other tumours of supposedly neuro-ectodermal <u>origin</u>, such as carcinoids (41,42,43,64) and oat-cell carcinomas of the lung (64 65 66) At least some pheochromocytomas seem to be able to produce immunoreactive calcitonin (40 41 48,67) Theoretically it seems quite plausible that pheochromocytoma closely related to MCT as part of the *familial chromaffinomatosis* (10), might be able to produce calcitonin or a calcitonin-like peptide
The raised levels of S-calcitonin reported in patients with the Zollinger-Ellison syndrome may be due to a constant C cell stimulation by the elevated S-gastrin as well as an ectopic synthesis of a hypocalcemic, calcitonin-like factor (68 69)

Elevated S-calcitonin levels were also recently reported in patients with carcinoma of the breast and non-oat cell lung cancer (44 45)

2/ Metabolic disturbances
Pathological conditions reported to be associated with high levels of S-calcitonin are hypercalcemia primary hyperparathyroidism, pernicious anemia chronic renal failure and pycnodysostosis. In all these conditions varying results of S-calcitonin determination have been reported (52 61 62 63 70 71) Raised levels are also seen in normal term babies and neonatal hypocalcemia (72 73)
One important factor which may cause diverging results is non-specific binding of antibody in the radioimmunoassay system. Here the argument of absorbability to charcoal has been considered very important to prove specificity (61) However recently it was shown that the addition of charcoal to serum introduces new artefacts as regards the binding capacity of antigen versus antibody (74) Furth rmore physicochemical methods do not allow absorption of all small polypeptides in serum (75)

Different results as regards meas red levels of immunoreactive calcitonin may actually be expected due to a number of factors:

1/ Antisera are diff rent as to specific binding affinity i e are directed against different antigenic determinants of the calcitonin molecule

2/ S-calcitonin shows an immunological heterogeneity in MCT It is possible that S-calcitonin may have a corresponding h terogeneity in other metabolic disturbances (76 77 78) In serum from patients with chronic renal f ilure gel chromatography with Sephad could separat out immunoreactive calcitonin which had a high r molecul r weight than synthetic calcitonin M (63)

3/ A number of tumours may produce peptid s which in certain fragments are similar t calcitonin and re ther fore bound to the antibody producing an elevation of S-calcitonin Thus for cl rity we do not eas re calcitoni but calcitonin-like activity in radioimmunoassay or immunoreactive calcitonin

At present we are investigating the clinical specificity of our method for S-calcitonin in diff rent conditions (non-MCT tumours and various metabolic dist rbanc s)

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up over several years will tell for certain whether the prognosis was improved or not The controls are those patients who had not been diagnosed and treated until they sought medical attention because of clinical symptoms of disease In this group only one out of nine patients was free from tumour according to S-calcitonin determinations Thes patients also must be followed up continuously for further comparison

However considering the numerous fatal cases reported in the literature our own experience and the survival rates reported for MCT I feel that a screening program for MCT is justified It seems probable that many of the individuals who are diagnosed and treated at an earlier stage do benefit
I am aware of the negative effects as well Not only the practical inconvenience of repeated controls but also the psychological burden of anxiety and uncertainty is laid on all the memb rs of the Sipple family including thos relative who will never develop the disease (82) This disadvantage can be less ed if a good relationship with personal continuity between patient and doctor is established

The survival rat f MCT is known to be int rmediat between th relatively good prognosis of papillar and follicular carcinoma and the very poor one of undifferentiated thyroid neoplasms (28) Fletcher (83) reported survival rates of 48% at 5 years after d finitive surgical treatment and 34% at 10 years. A better survivorship was recently reported by Chong et al.(84): 80 and 67% respectively
In the individual case however th atural course of MCT seems rather unpredictable Certainly there are patients in whom the tumours evidently behave lik benign tumours as reported by Ljungberg (85) and s seen in some of o r patie ts On the other hand we find that everal of the relati s f our Sipple patients have died of generalised MCT in spite of treatment. In the lit rature there are exampl s of extremely early spread to lymph nod s g in a clinically asymptomatic 4½-year-old boy who was subjected to prophylactic" thyroidectomy because of heredity for MCT (86) Dunn t al (87) hav r ported clinically apparent MCT in two children aged 6 and 7 in a Sipple family as well as transformation f MCT into a highly malignant anaplastic form

How specific is the C cell response to hypercalcemia? Normal and neoplastic C cells possibly respond to physiological stimuli in the same way But, we know only little about the reactivity of other neoplastic cells with a production of calcitonin, irrespective of their possible ontogenetic relationship to C cells, i e *derivation from the neural* crest In paper III we presented the hypothesis that *non-MCT tumours with ectopic production of calcitonin* would not respond to physiological stimuli in the same way as normal C cells If so we might be able to differentiate between MCT and non-MCT tumours with ectopic calcitonin production In 1974 Coombes (79) reported that some non-MCT tumours released calcitonin after hypercalcemia Evidently more studies are needed to solve this question

Hypocalcemia causes a storage of calcitonin in rat C cells (80) We could not find evidence of increased calcitonin storage in two patients with chronic hypocalcemia as expressed in increased ΔCT_{240} (paper III) Deftos (81), *however, observed an increased secretion of calcitonin in* response to hypercalcemia in patients with hypocalcemia The conflicting results may be explained by the different sensitivity of the radioimmunoassay methods for S-calcitonin determination or that too few patients were tested in our study

Now, having evaluated the <u>tools</u> for diagnosing MCT, we must consider the <u>clinical</u> end <u>results</u> of the screening program

II Evaluation of the clinical results of the screening program
Aims of the investigation, motivation and fulfillment

My practical clinical goal was the early diagnosis of MCT and pheochromocytoma in all individuals carrying the genetic trait for Sipple s syndrome My hope was that earlier diagnosis and treatment would improve the prognosis for the individual Therefore I wanted to institute treatment for MCT before an inoperable stage was reached or the patient had suffered cardiovascular catastrophies due to overlooked pheochromocytoma

According to Sackett (39) screening must lead to an improvement in end-results among those in whom early diagnosis is achieved It is still too early to judge the final end-results of my screening program Only a follow

recognised the early or preneoplastic stages of MCT are still less well defined.
The designation occult tumour means that the patient is asymptomatic and the tumour diameter is less than 15 mm (28) C cell hyperplasia is defined only by Wolfe et al. (27) as a very early stage of C cell proliferation when macroscopic tumours are not yet to be seen According to their definition all our MCT patients really had MCT and not C cell hyperplasia
An extended histopathological definition of the early stages of MCT would be of value taking into consideration criteria such as nuclear anisocaryosis irregularity of C cell distribution in the thyroid tissue and invasiveness.

What were the practical consequences for the screened individuals i e did the positive diagnosis of tumour lead to treatment? This is essential in evaluati g the clinical result of a screening program. Th diagnosis was of practical consequence in 15 of 17 patients. Fourteen patients have been operated on for MCT as soon as a definitive diagnosis was established One patient is awaiting surgery One is being followed elsewhere and one patient does not want treatment
However in the patients earli r op rated upon (non-radically) the diagnosis was of much less practical importance So far only one of them has been reoperated In another two furthe ff rts to localise metastases are considered eaningful Probably they will be subjected to s lective venous cath terisation with regional analysis of serum calcitonin (The results of this diagnostic method will b reported separately (89))
(Seven ew cases of pheochromocytomas wer diagnosed Five of thes have been operated as co sequenc f the diagnosis One patient does not want s rgical treatment and one is judged inoperable due t coronary heart disease)

Will th indi idual prognosis be improved by ea li r diagnosis and treatment? Can a p limi ry judgem nt be mad regardi g t end-result f scr eni g for MCT? (Se Sockett di cus on abov Th rognosis of a alignant tumour i gen rally correl ted t t cli cal stag of th diseo i e th bsence or presence f l al distant t st s s
MCT is known to metastasize early (8ɔ 9ι although w do not know if ther is a diff renc between th dι and th h editary variety

The occurence of concomitant pheochromocytomas may create a greater threat to life than MCT (88), as exemplified also by the index case in family I Some of our patients have had symptoms of pheochromocytomas for many years without recognition of the true cause of these symptoms As illustrated by Steiner in his extensive patient review (11) the pheochromocytomas are a common cause of death among Sipple patients Thus, one of the most important aspects of identifying the Sipple genome carrier at an early age is the possibility to diagnose and treat the pheochromocytomas in time

How efficient was our screening program for MCT? Could we diagnose as many cases as was theoretically expected? The expected frequency would be about 50% due to the autosomal-dominant trait with high penetrance of the gene
Actually we found 17 new cases of MCT in 49 individuals (= 35%), which together with the 9 patients earlier treated gives 26/58 (= 45%) However 4 of our subjects were children and these were not subjected to our best diagnostic methods i e the stimulation tests Thus more cases will probably be diagnosed in the future The observed frequency is therefore regarded as a satisfying result

Does screening for MCT lead to earlier establishment of the MCT diagnosis as compared with the old methods, i e clinical examination and thyroid scintiscanning? Seventeen new MCT cases were found Despite careful examination 11 patients had completely normal palpatory findings Thyroid scintigraphy was a better method Some patients had clearly abnormal pictures with well delineated areas of reduced uptake, and some only a slight irregularity in the distribution of the isotope However the interpretation of the scintigrams was biased by the fact that in many cases the result of the determination of serum calcitonin was known at the time of examination (The results (37) of a more extensive study with different isotopes in MCT will be presented separately) However it can be concluded that scintigraphy was less sensitive than the new methods for the correct prediction of MCT as normal scintigrams could be found in patients with very small tumours of only millimeter size
Screening with the new methods thus leads to earlier establishment of the MCT diagnosis (Fig 1) Whereas the histopathological basis of the MCT diagnosis is well founded and

GENERAL SUMMARY AND CONCLUSIONS

A/ Aim of the investigation

I have studied the possibilities for diagnosis of early occult *medullary carcinoma of the thyroid* (MCT) *especially in families* with Sipple s syndrome (hereditary MCT combined with bilateral pheochromocytomas)

B/ Diagnostic methods

1 *The following new* diagnostic methods have been developed and evaluated:

a/ Fin needle aspiration biopsy with cytological examination (paper I)

b/ A radioimmunoassay for human serum calcitonin (S-calcitonin) (to be used in c/ and d/)

c/ Determination of basal i e non-stimulated serum calcitonin (pap r II)

d/ Determination of the serum calcitonin response to stimulation with induced hypercalcemia (calcium infusion test) pentagastrin cholecystokinin-pancreozymin and thanol (paper III and IV)

2. The new methods have been compared with each oth r and with the old r ethods used for clinical diagnosis of thyroid neoplasms:

/ Physical examination with caref l palpation of the thyroid by at lea t two xperienced physicians

b/ Thyroid scintigraphy using ^{131}I and/or ^{99m}Tc-p rtechnetate

C/ P tient material

Th diagnostic m thod have been applied to 59 members of 4 s parate families with Sippl s syndrome 12 patients with sporadic MCT as well as to healthy subject and patients with other di eas s than MCT who s rved a controls

D/ Diagnosti results of each m thod:

I: Fine eedl spiration biopsy

Eight en patients with MCT were e amined Th technique was impl e d quick causing egligible discomfort to the patient No complications were een Th typical MCT c ll had cha ct ristic appearance

The results of the Boston group (91) are of great interest: In 7 out of 12 patients operated on for occult MCT local lymph node metastases were found As in our cases of occult cancer the diagnosis was established with calcium infusion test and determination of serum calcitonin The incidence of histopathologically verified local metastases in our patients is lower (2 out of 14) According to the results of postoperative S-calcitonin determinations 5 patients did have metastases (local or distant), which obviously could not be recognised by the surgeon at the operation

Only many years follow up will tell for certain whether our preliminary opinion of postoperative radicality is correct (9 of 14 of the MCT patients found during screening, as opposed to 1 of 9 in the earlier operated group which however, has a much longer follow up period)

In one of our patients non-radicality could not be demonstrated until 5 years after surgery During these years the normal results of calcium infusion tests were obviously "false negative Apparently the biological behaviour of this very tumour and its metastases seems relatively benign

However one conclusion may be drawn from these preliminary results: Diagnosis must be established at a still earlier age in order to increase still more the chances for radical cure Stimulation tests must be performed also in the small children Support for this opinion was recently obtained by Chong et al (84), who reported a 2-year-old child with C cell hyperplasia diagnosed by a similar type of family screening (For a discussion of MCT as a congenital tumour or C cell hyperplasia as a preneoplastic stage see paper III)

All 5 have si ce shown rising ΔCT_{240} into the range for MCT
4 have been verified by surgery and had tumours less than 5 mm in diameter
Thus for the early diagnosis of occult MCT i.e very small asymptomatic tumours the calcium i f sion test was shown to be a more sensitive method than determination of basal S-calcitonin
No false positives were seen

IV Serum calcitonin response to pentagastrin chol cystokinin-pancreozymin and ethanol

In order to simplify the use of stimulation tests for screening Sipple families pentagastrin cholecystokinin-pancreozymin and ethanol were tried as stimulators of calcitonin release
Pentagastrin s c. or i.v induced a pronounced a d rapid increase of S-calcitonin within 2 — 5 min The elevation was roughly proportional to the tumour mass as estim ted at operation Seventeen of 18 patients ith MCT (all but one clinically occult) responded to s c. pentagastrin ith a significant increase in S-calcitonin which correlated well to that induced by th calcium infusion test In the single non-responder i v pentagastri induced a rise in S-calcitonin equivalent to that in the calcium infusion test I.v pentagastrin induced more pronounced elevation of S-calcitonin than did s.c. pentagastrin whereas no difference was seen in the control group The pentagastrin test was found to be much simple and quick r than the calcium infusion test which is both laborious and tim consumi g (continous patient surveillance is needed during the 4 hour inf sion): only two blood sampl s re needed one just before the i v or s c injection of pentagastrin and the oth r 2 or 5 min after The sensitivity of the calcium infusion test and pentagastrin test were pproximately equal. Cholecystokinin-pancreozymin and ethanol had no advantages over pentagastri as the S-calcitonin response was weaker

V Physical xamination and thyroid cintigraphy

Thyroid scintigraphy was a more sensitive method than physical examination: In 8 of 15 new cases of MCT th scintigram was clearly abnormal i.e indicating one or several tumours. In 4 patients the scintigram was judged as uncertain and in 3 patients as normal

in May-Grünwald-Giemsa stain: a triangular cell with one or several eccentrically positioned nuclei and a distinct red granulation
Lumps of amyloid usually in an extracellular position showed green birefringence in polarised light after staining with alkaline Congo red
Correct diagnosis of MCT was achieved in 12 of 13 patients with a palpable thyroid and/or lymph node abnormality, but only in 1 of 5 without a palpatory target to guide the puncture needle

II Determination of S-calcitonin

A radioimmunoassay for determination of human S-calcitonin was developed The assay was specific for human synthetic calcitonin M and native calcitonin in serum from patients with MCT
Reference range in healthy controls (adults and children) was 0 3 - 1 0 ng/ml High S-calcitonin levels 2 - $>$800 ng/ml were found before surgery in all 21 patients with verified MCT, and low levels in 7 out of 9 patients after thyroidectomy for thyroid tumours other than MCT Only 16 of the 21 MCT patients had a palpable neck abnormality i e determination of S-calcitonin had a higher sensitivity than physical examination
The levels of S-calcitonin correlated well with the clinical estimation of the extent of the disease No false positives were seen in this patient material

III Serum calcitonin response to induced hypercalcemia

Transient hypercalcemia was achieved by a 4 hour infusion of calcium gluconate The change in S-calcitonin induced in this way was called ΔCT_{240}.
Reference range for ΔCT_{240} was -0 2—+0 5 ng/ml High values 2.2—630 ng/ml were found in all 13 MCT patients However in these cases the diagnosis was already evident from the basal level of S-calcitonin
In 5 out of 19 clinically healthy first degree Sipple relatives (with normal or only slightly raised (i e $<$2 ng/ml) basal S-calcitonin) ΔCT_{240} was slightly elevated intermediate between the controls and the MCT patients These 5 patients were called the borderline group

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On the other hand physical examination revealed a palpable thyroid abnormality in only 6 of the 17 new cases of MCT Thus, physical examination performed even by different physicians especially experienced in thyroid diseases, proved to be the least sensitive method for diagnosing early MCT

E/ Clinical results

Seventeen new cases of MCT were diagnosed as a result of the screening of the Sipple families None of these patients had sought medical attention because of any symptoms referring to the thyroid gland Up to now 14 of these patients have been operated and the diagnosis verified by histopathology Most of these patients were young (median age 27 years) Tumour size (as measured at operation) was more than 10 mm in 8 patients, between 5 and 10 mm in one and less than 5 mm in 5 Result of treatment The provisional judgement regarding radicality was based on histopathological examination of the surgical specimen and the result of S-calcitonin determinations during stimulation with calcium infusion or pentagastrin Follow up time varied from 5 years to a few months At the conclusion of this study 9 of 14 patients were considered as radically treated and the other 5 patients had residual tumour growth Correlation was found between radicality of treatment and age (at the time of diagnosis) as well as tumour size (as estimated at physical examination i.e palpable or non-palpable tumour and as measured at operation) Thus it was shown that the chance for radical cure was greater in the youngest patients who had also the smallest tumours

These results of treatment were compared with those in 9 patients, who had previously been operated for familial MCT (all of them after appearance of clinical symptoms and signs of tumour) Only 1 of 9 was free from residual tumour growth according to the results of S-calcitonin determinations However these groups are not quite comparable as the time from operation to that at last reexamination was longer (range 2 — 13 years) than in the first group Patient age at the time of diagnosis was also higher than in the group of patients detected through the screening program (median age 42 years)

F/ Conclusions

1 Sipple screening pays
Seventeen out of 49 Sipple relatives screened had MCT (35%) Eighteen of the relatives provisionally classified as healthy were children and young adults (less than 30 years old) Some of these had probably not yet developed tumours large enough to be detected even by sensitive tests

2. Follow up of patients earlier operated for familial MCT showed that only very few of these had been radically treated. They had then been treated only after they had developed symptoms of neoplastic disease

3 With the new diagnostic methods MCT could be diagnosed earlier when the tumour was still only a few millimeter in size It was shown that the chance for radical treatment is greater if the tumour is diagnosed at an early stage The patients may then be clinically asymptomatic i.e. feel well and have normal findings at physical examination and thyroid scintigraphy
These m thods offer the following therapeutic advantages:
/ surgical therapy can be offered earlier
b/ the operation can be made less traumatizing i.e performed with less risk for complications and with l ss need for extension of the operatio to radical neck dissection and mediastinal lymph node evacuation

4 The family history was not enough to cl ssify a ew case of MCT as sporadic or hereditary It had to be supplemented with investigation of the relatives.

5 My experienc in this study of th new diagnostic methods may be summed up s follows:
In patie ts with suspected MCT d termination of S-calcitonin (the basal level and that fter administration of pentagastrin) will verify the presenc f even very small occult tumours In screeni g f r hereditary MCT this method should be applied regardless of th possibl presence f a goiter
I patients with s spected MCT nd a palpable neck abnormality fi needle spiration biopsy can be recommended as simpl and quick m thod with good sensitivity and specificity

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MCT histopathological diagnosis: Defined according to Hazard et al. (2) as opposed to "C cell hyperplasia as described by Wolfe et al (27)

MEA: Multiple endocrine adenomatosis.

MGG: May-Grünwald-Giemsa stain

PG: Pentagastrin.

Pheochromocytom clinical diagnosis of in Sipple relatives: Positive biochemical evidence of increased cathecholamine production and/or uni or bilateral adrenal tumours at selective suprarenal arteriography and phlebography

Pheochromocytoma scr ening for: Clinical examination + repeated determinations of dU-VMA dU-methoxycatecholamin s and dU-cathecholamines

Radicality of surgical treatment: Judged from a/ histopathological examination of the surgical specimen and b/ results of determination of S-calcitonin after stimulation tests

S-: Serum.

Screening: T sting of apparently healthy s bj cts f r th purpose of separating them into groups with high and low probabilities for a given disorder e g in order to initiate a proces of early diagnosis and treatment (See ref 39)

Sippl s syndrome: An autosomal dominant hereditary disease with bilateral medullary carcinoma of th thyroid often in combination with bilat ral pheochromocytomas I the familial cases I hav used the t rm Sipple s syndrome and Sipple patient even if the syndrome is not complete in th actual patient

Sippl genome: Genome for Sippl s syndrome

Sippl pati t: Patient with either MCT and/or pheochromocytoma who is ember of a Sipple family

Sippl relative: In most cas s a first degree r lative of a Sipple patient.

Sippl elativ healthy: Provisi ally regarded as healthy i e at p ese t free from d tectabl MCT according to the result of the screening examination Another de ignation is high risk individual (in paper IV)

VMA: 4-hydroxy-3-methoxymandelic acid

Unl s oth rwis specified ref renc s to tables pedigrees and figure ref r to this ummary

DEFINITIONS AND ABBREVIATIONS

Borderline patients: Symptomless Sipple relatives, classified on the basis of the results of the calcium infusion test The values of the classifying parameter ΔCT_{240} is intermediate between that for the healthy controls and that for verified MCT patients

Calcitonin basal level: The non-stimulated level of S-calcitonin, i e before release of calcitonin has been induced by calcium infusion, pentagastrin or other stimulating agents Usually the blood sample was taken in the morning from the fasting patient

CT: Calcitonin

ΔCT (delta-calcitonin): The change in S-calcitonin induced by stimulating agents such as calcium infusion ($\Delta CT_{240} = \Delta CT_{Ca}$), pentagastrin ($\Delta CT_{PG}$), cholecystokinin-pancreozymin (ΔCT_{CCK} and ΔCT_{C8-CCK} resp) and ethanol (ΔCT_{Et})

Calcium infusion test: A standardised i v infusion of 0 375 mmol calcium as the gluconate during 4 hours The induced hypercalcemia gives a change in S-calcitonin which is measured from basal level to that at the end of the infusion This change is denoted ΔCT_{240} (paper III and this summary) or ΔCT_{Ca} (paper IV)

CCK: Cholecystokinin-pancreozymin (the native porcine extract)

C8-CCK: The synthetic C-terminal octapeptide of porcine cholecystokinin-pancreozymin

Clinical examination: Patient history and physical examination

Cytology: Sometimes used as a short synonym for fine needle aspiration biopsy with cytological evaluation"

dU: Urine collected during 24 hours

MCT: Medullary carcinoma of the thyroid

MCT hereditary: MCT as part of Sipple s syndrome

MCT sporadic: MCT without evidence of being part of Sipple s syndrome

MCT, occult: Subclinical tumour below 15 mm in diameter (see ref 28)

MCT histopathological diagnosis: Defined according to Hazard et al (2) as opposed to "C cell hyperplasia as described by Wolfe et al (27)

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Sippl genom : Genom for Sipple s syndrom

Sippl patient: Patient with either MCT and/or pheochromocytoma who is memb r of Sipple family

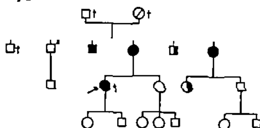
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Sippl rel tive healthy: Provision lly regarded as h althy i. at pre ent free from d t ct ble MCT according to the result of the screeni g examination Anoth r d signation i high risk individual (in paper IV)

VMA: 4-hydroxy-3-metho ymandeli acid

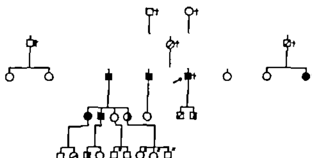
Unl ss otherwis specified references to tables pedigrees and figure ref r to this summary

Family I



Medullary Carcinoma of the Thyroid
Phaeochromocytoma
Symptoms/signs or history suggestive of the syndrome
Not examined
Indicates the proband of the family

Family II

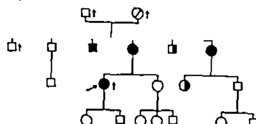


Pedigrees for the Sipple families I and II (Patient Her:3 in Family II is represented by the black square to the far left in generation 3)

T ol I RESULTS OF SCREENING FOR HCT AND PHEOCHROMOCYTOMA IN FOUR FAMILIES WITH SIPPLE S SYNDROME

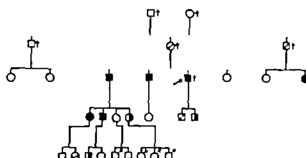
	Family				
Number of subjects	I	II	III	IV	Total umber
Number of individuals xamined in each family (Within brackets number of patients previously treated for HCT and now eexamined)	12(3)	17(0)	16(4)	13(2)	58(9)
Number of ew cases of HCT d tected by the screening program (Within brackets umber of earlier operated cases now found to be non-radically treated.)	2(2)	7(0)	5(4)	3(2)	17(8)
Number of patients previously operated for pheochromocytoma		1	2		3
Number of ew case of pheochromocytoma de- t cted by the screening program	3	3	1		7

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Not examined
Indicates the proband of the family

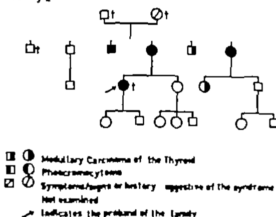
Family II

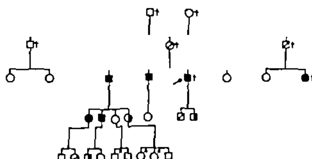


Pedigrees for the Sipple families I and II (Patient Her:3 in Family II is represented by the black square to the far left in generation 3)

T bl I RESULTS OF SCREENING FOR MCT AND PHEOCHROMOCYTOMA IN FOUR FAMILIES WITH SIPPLE S SYNDROME

	Family				
Number f subjects	I	II	III	IV	Total umber
Number of individuals examined in each family (Within brackets umber of patients previously treated for MCT and now reexamined)	12(3)	17(0)	16(4)	13(2)	58(9)
Number of new cas s of MCT detected by th screening program (Within brackets number of earlie operated cas s now found to be non-radically treated)	2(2)	7(0)	5(4)	3(2)	17(8)
Number of patients previously operated for pheochromocytoma		1	2		3
Number of new cases of pheochromocytoma detected by the screeni g program	3	3	1		7





Pedigrees for the Sipple families I and II (Patient Her:3 in Family II is represented by the black square to the far left in generation 3)

T ble III EARLIER OPERATED CASES OF HEREDITARY MCT: FINDINGS AT LAST REEXAMINATION

Patient	Se	Ag t first operation	Age	S bjective ymptoms	Palpable tumour	S-cal toni: Basal	Post- timulation ΔCT_{240} and/or ΔCT_{PG}
No : 18	F	48	55	0	0	Raised	Raised
19	M	51	56	0	0	Normal	Normal
20	F	39	52	0	0	Raised	Raised
21	M	50	59	0	0		
22	M	48	57	0	0		
23	F	39	48	0	0		
24	F	36	42	0	0		
25	F	42	44	0	0		
26	M	42	44	0	0		
T tal: M 4 F 5		Mean 44 years				Normal 1/9 Raised 8/9	Normal 1/9 Raised 8/9

Table II NEW CASES OF MCT RESULTS OF PHYSICAL EXAMINATION SCINTIGRAPHY CYTOLOGY AND HISTOPATHOLOGY

Patient	Sex	Age at diagnosis	Physical findings	Scintigraphy	Cytology	Histopathology
Mars 1	M	56	Nodular goiter+Mlp	Abnormal	MCT	Verified by histopathology
2	F	22	Nodular goiter+Mlp	Abnormal	MCT	*
3	M	58	None	Normal	Biopsy material insufficient	Refuses surgery
4	M	57	Nodular goiter	Abnormal	MCT	Verified by histopathology
5	M	33	Nodular goiter	Abnormal	MCT	
6	F	30	None	Uncertain	Normal	
7	F	35	Nodular goiter	Normal	Normal	Followed elsewhere
8	M	25	None	Uncertain	Normal	Verified by histopathology
9	M	15	None	Uncertain	Not performed	
10	F	21	None	Uncertain	Not performed	
11	M	21	None	Abnormal	MCT	
12	M	29	None	Abnormal	Normal	
13	M	28	None	Not performed	Not performed	Awaiting surgery
14	F	21	None	Normal	Normal	Verified by histopathology
15	M	25	None	Abnormal	Normal	*
16	F	27	None	Abnormal	MCT	*
17	F	26	Nodular goiter	Uptake too low for evaluation	Performed elsewhere abnormal	
Total	M 10 F 7	Mean 31 years	Nodular goiter 6/17 No palpable abnormality 11/17 Mlp = Marfan-like physique	Abnormal 8/15 Normal 3/15 Uncertain 4/15	Biopsy material sufficient for evaluation 12* MCT 6 Normal 6	Total thyroidectomy performed in 14 patients MCT was verified in all 14

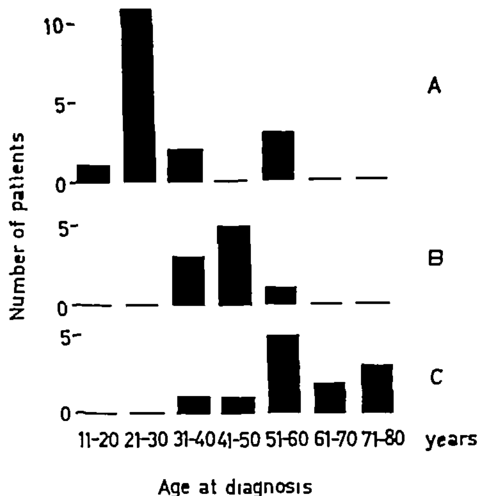


Figure 1 INCIDENCE BY AGE IN HEREDITARY AND SPORADIC MCT

A. Familial cas s detected by the screening program (mean age 31 years median age 27 years)

B Famili l c ses diagnosed after manif station of clinic l symptoms (mean age 44 years median age 42 years)

C. Sporadic cases diagnosed aft r manifestation of clinical symptoms (mean ag 58 years median age 57 years)

Table IV RELATIONSHIP BETWEEN CLINICAL FINDINGS AND RESULTS OF SURGICAL TREATMENT IN 14 MCT PATIENTS DIAGNOSED BY THE SCREENING PROGRAM

	Clinical parameter for comparison	Number of radically treated patients	Number of non-radically treated patients	Total number of patients
A	Patient age at first operation			
	11 - 20 years	1		1
	21 - 30	7	3	10
	31 - 40	1		1
	41 - 50			
	51 - 60		2	2
B	Physical examination of the thyroid and/or lymph nodes of the neck			
	No palpable abnormality	8	1	9
	Palpable abnormality	1	4	5
C	Tumour size at operation (diameter of largest tumour)			
	≤ 5 mm	5		5
	> 5 - ≤ 10 mm	1		1
	> 10 mm	3	5	8

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